A Data Based Perspective on the Environmental Risk Assessment of Human Pharmaceuticals

Thesis

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A Data Based Perspective on the Environmental Risk Assessment of Human Pharmaceuticals

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ABSTRACT

The EU Medicinal Products Directive (65/65/EEC) has been amended to require an environmental risk assessment (ERA) for human pharmaceuticals effective January 1995. At present, official ERA guidelines have yet to be finalised. Previous discussions about their nature have taken place in the absence of a systematic analysis of the potential environmental impacts of pharmaceuticals. This study attempts to address this deficiency via a review of existing ecotoxicity data. Acute ecotoxicity data relating to >100 human pharmaceuticals have been collated. They suggest a lack of acute effects at <100 µg/l in standard tests. Relative sensitivity based on acute effects was algae (most sensitive) > *Daphnia* > fish. Chronic effects data were limited and this was identified as a shortcoming. This was reinforced by observations of large differences between acute and chronic responses to steroids in fish. The availability of UK usage data permitted risk characterisation i.e., calculation of PEC/PNEC[1] ratios for >60 compounds. Under "*worst-case*" fate assumptions of no human metabolism, passage of all material to drain, no removal during wastewater treatment and no surface water dilution of effluent, the large majority of pharmaceuticals yielded PEC/PNEC ratios <1 (in theory implying environmental safety). For the remainder, a consideration of surface water dilution and expected wastewater treatment removal was sufficient to yield PEC/PNEC <1. PNEC was based on acute effects data with an assessment factor of 1,000. These assessments ignore the potential for multiple exposure/mixture effects. Calculation of potential lifetime ingestion via drinking water employing "*worst-case*" assumptions (as above and no removal during drinking water treatment) revealed I_{70} values (based on ingestion of 2 litres/day for 70 years) generally equivalent to ≤2 days of the corresponding daily therapeutic doses. Refinement of the exposure calculations or comparisons with monitoring data confirmed the degree of conservatism associated with the "*worst-case*" exposure estimations.

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1 Predicted Environmental Concentration (PEC) and Predicted No-Effect Concentration (PNEC).

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I would like to acknowledge the guidance and patience of my supervisors Tom Feijtel (Procter & Gamble) and Roger Hill (Open University).

DECLARATION

None of the material submitted has previously been submitted for a degree or any other qualification at any university or other institution. The author is the sole contributor to the work. Various parts of the thesis have been published in 2001. This is clearly indicated within the text of the thesis.

DEDICATION

This thesis is dedicated to the memory of my late father Cyril William Frederick Webb (1927 - 2000).

CHAPTER 1

INTRODUCTION

INTRODUCTION

There is a growing literature in relation to observations of human pharmaceuticals in the environment, in particular from sewage and surface waters. These have been reviewed elsewhere (Daughton & Ternes, 1999; EA, 2000). Discussions about the environmental consequences (i.e., safety or risk) of the presence of such compounds have taken place in the general absence of a systematic analysis of the potential risk, with only a few notable exceptions (Halling-Sørensen *et al.*, 1998; Stuer-Lauridsen *et al.*, 2000). This can partly be attributed to the lack of public domain information relating to the ecotoxicity of pharmaceuticals. Concurrently, there have been various regulatory developments in the USA and EU relating to requirements for environmental risk assessment (ERA) of new drug actives as part of their registration process. The lack of such an analysis means that to date, decisions concerning ERA criteria have been somewhat arbitrary or based on comparisons with inappropriate groups of industrial chemicals such as pesticides. This study aims to address that deficiency and collates examples of data relating to the environmental fate/concentration (Chapter 2) and ecotoxicity (Chapter 3) of existing pharmaceuticals. Where possible, these data are employed in environmental risk assessments of compounds in the aquatic compartment (Chapter 4). Quantitative "*worst-case*" estimates of indirect human exposure via drinking water are similarly benchmarked against potential effects endpoints such as daily therapeutic dosage (Chapter 5). These elements and others are considered further in the General Discussion (Chapter 6). The overall intention is to provide perspective that will prove useful during the further development of regulatory assessment criteria.

REFERENCES

Daughton CG, Ternes TA (1999) Pharmaceuticals and personal care products in the environment. *Environmental Health Perspectives* 107 (Supplement. 6): 907-938.

EA (2000) *Review of Human Pharmaceuticals in the Environment.* R & D Technical Report P390 (Ayscough NJ, Fawell J, Franklin G & Young W). Research Contractor WRc_NSF Ltd. Environment Agency.

Halling-Sørenson B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten-Lützhøft HC, Jørgensen SE (1998) Occurrence, fate and effects of pharmaceutical substances in the environment - A review. *Chemosphere* 36(2): 357-393.

Stuer-Lauridsen F, Birkved M, Hansen LP, Holten Lützhøft HC, Halling-Sørensen B (2000) Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. *Chemosphere* 40(7): 783 – 793.

CHAPTER 2

FATE & EXPOSURE DATA

INTRODUCTION

An increasing number of human pharmaceuticals have been reported in the environment (e.g., Stumpf *et al.*, 1996; Heberer *et al.*, 1997; Ternes, 1998; Hirsch *et al.*, 1999). Data relating to observations in sewage, surface waters, groundwater and drinking water are collated here. Similarly, data influencing the fate of pharmaceuticals in the environment such as physico-chemical properties, biodegradation profile or fate during wastewater treatment have also been collated. An extensive discussion of fate and observed environmental concentrations *per se* will not be undertaken here. This has adequately been dealt with elsewhere (e.g., EA, 2000). The main thrust of this study is the benchmarking of predicted and observed concentrations against available ecotoxicity data. The data in this chapter have been collated to assist in this purpose.

PHYSICO-CHEMICAL DATA

The physico-chemical characteristics of pharmaceuticals help to determine their fate, effects and bioaccumulation. Of particular importance in this respect are the molecular weight (MW), the octanol/water partition coefficient (K_{OW}) and the acid dissociation constant (pK_a). Details of Chemical Abstracts Service numbers (CAS #), molecular weight, octanol/water partition coefficients (as log K_{OW}) and dissociation constants (Table 1).

The octanol/water partition coefficient (K_{OW}) is a surrogate measure of hydrophobicity/lipophilicity. Hydrophobicity in turn helps to determine adsorption to organic solids (Karickhoff 1981), ecotoxicity (Clements *et al.*, 1993; Kaiser & Esterby, 1991; Könemann, 1981) and bioconcentration/bioaccumulation (Mackay, 1982; Veith & Kosian, 1983; Geyer *et al.*, 1991). The acid dissociation constant (pK_a), together with ambient pH, determines the extent to which medicinal compounds exist in the unionised or ionised form. When the pK_a of a drug and

the pH of the aqueous phase is known, the proportion of ionised/unionised moieties can be calculated via the familiar Henderson-Hasselbach equations (see Bowman & Rand, 1980).

Table 1. - Physico-chemical characteristics of selected pharmaceuticals [from Bowman & Rand (1980); Dollery (1991); Hansch *et al.*, (1995); Newton & Kluza, (1978); Raymond & Born (1986); Hoekman, (1997)].

Compound	CAS #	MW	log K_{ow}[2]	pK_a[3]
Acarbose	56180-94-0	645	2.40	5.1
Acriflavine	86-40-8	224.27	-1.78	
Alendronic Acid	66376-36-1	249.1		
Aminosidin/Neomycin E	7542-37-2	615.56		
Amitriptyline	50-48-6	277.44	5.04*	9.4c
Amobarbital	57-43-2	226.27	2.11/2.07	7.94a
Amopyroquin	550-81-2	353.83	2.57 (7.4)	
Amphetamine	300-62-9	135.21	1.76*	9.8c
Aprotinin	9087-70-1	6500		
Aspirin	50-78-2	180.17	1.19*	3.5a
Atropine	55-55-8	289.38	1.83*	9.9c
Azithromycin	83905-01-5	748.99		
Bacitracin	1405-87-4	1422.59	-0.92	
Bicalutamide	90357-06-5	430.4		
Budesonide	51333-22-3	430.55	3.28	

[2] Preferred values from Hansch *et al.* (1995) are denoted by an asterisk. Values in parenthesis denote the pH at which K_{ow} was determined.

[3] Anion forming (i.e., acidic) denoted by a and cation forming (i.e., basic) by c.

Caffeine	58-08-2	194.22	-0.07*	0.6 c, 13.9a
Carvedilol	72956-09-3	406.48		
Cefprozil	92665-29-7	389.4		
Ceftibuten	97519-39-6	410.42		
Cetirizine	83881-51-0	388.89	1.70*	
Chloramine T	127-65-1	227.67		
Chloramphenicol	56-75-7	323.15	1.14*	5.5
Chloroquine	54-05-7	319.89	4.63*	8.4c, 10.8c
Cimetidine	51481-61-9	252.34	0.40*	6.8c
Cisapride	81098-60-4	465.95		
Cladribine	4291-63-8	285.69		
Clofibric Acid	882-09-7	214.66	2.57*	2.95a
Cyclophosphamide	50-18-0	261.10	0.63*	
Cyclosporin A	59865-13-3	1202.6	2.92 (7.4)	
Dextropropoxyphene	469-62-5	330.48	4.18 (9.2)*	6.3c
Diazepam	439-14-5	284.76	2.99 (7.4)*	3.2c
Didanosine	69655-05-6	236.2	-1.24 (7.0)	
Diethylstilbestrol	56-53-1	268.34	5.07*	
Digoxin	20830-75-5	780.92	1.26*	
Dirithromycin	62013-04-1	835.1		
Dorzolamide	120279-96-1	324.4		
Erythromycin	114-07-8	733.95	2.54 (8.0)*	8.8c
Ethinyl Oestradiol	57-63-6	296.41	3.67*	10.5
Famciclovir	104227-87-4	321.3		
Famotidine	768224-35-6	337.43	-0.57 (9.2)	7.1

Finasteride	98319-26-7	372.55	3.03	
Flumazenil	78755-81-4	303.29	1.15	1.7
Flumequine	42835-25-6	261.26	1.11 (7.2)	
Fluoxetine	54910-89-3	309.3	1.82 (7.4)	
Fluticasone	80474-14-2	500.6		
Gabapentin	60142-96-3	171.2		
Ibuprofen	15687-27-1	206.27	3.50(2.0)*	5.2a
Ifosamide	3778-73-2	261.07	0.86*	
Iopromide	73334-07-3	791.12	-2.33	
Isoniazid	54-85-3	137.16	-0.70*	2.0c, 3.85c
Ketoprofen	22071-15-4	254.29	2.76	
Ketorolac	74103-06-3	255.27	2.72	3.54
Lansoprazole	103577-45-3	369.4		
Lincomycin	154-21-2	406.56	0.20*	7.6c
Lithium Carbonate	554-13-2	73.89		
Lithium Citrate	6080-59-6	281.99		
Lomefloxacin	98079-51-7	351.35	-0.80*	
Loracarbef	76470-66-1	349.8		
Losartan	114798-26-4	461.0		
Methotrexate	59-05-2	454.47	2.28	
Metformin	657-24-9	129.17	-1.43	2.8c, 11.5
Metronidazole	443-48-1	171.16	-0.02*	2.62c
Midazolam	59467-70-8	325.77	1.53(7.4)	6.2
Milrinone	78415-72-2	211.22		
Naproxen	22204-53-1	230.26	3.24 (2.0)*	4.39a

Nefazodone	83366-66-9			
Nicotine	54-11-5	162.23	1.17(11.0)*	3.2c, 8.0c
Nitrofurazone	59-87-0	198.14	0.23 (7.4)*	
Omeprazole	73590-58-6	345.42	2.23*	4.0, 8.7
Ondanestron	103639-04-9	293.37		7.4
Orphenadrine	83-98-7	269.36	3.77*	9.0c
Oxolinic Acid	14698-29-4	261.2		
Oxytetracycline	79-57-2	460.44	-0.89 (5.5) -0.92 (6.6) -1.60 (7.5)	3.3c, 7.3a, 9.1c
Paclitaxel	33069-62-4	853.9		
Paracetamol	103-90-2	151.18	0.51 (2.0)*	9.5a
Paroxetine	61869-08-7	329.37		
Perindopril	82834-16-0	368.47		
Pentobarbital	57-33-0/ 76-74-4	226.27	2.07*/2.10*	8.11a
Phenobarbital	50-06-6	232.26	1.47 (2.0)*	7.2a
Porfirmer Sodium	87806-31-3			
Propranolol	525-66-6	259.35	2.98 (10.2)*	9.5c
Quinacrine	83-89-6	399.93	1.91 (7.4)	
Quinidine	56-54-4	324.43	2.88 (7.4)*	4.2c, 8.8c
Quinine	130-95-0	324.43	2.64 (7.4)*	4.1c, 8.5c
Ranitidine	66357-35-5	314.41	0.27(10.5)*	2.7c, 8.2c
Risperidone	106266-06-2	410.5		
Salicylic Acid	69-72-7	138.12	2.26*	3.0a, 13.4a

Salmeterol	89365-50-4	415.8		
Secobarbital	76-73-3	238.3	1.97	7.92a,12.60a
Simethicone	8050-81-5	15k - 26k	>12 .5[4]	
Spirapril	83647-97-6	467.1	-1.10(7.4)	
Stavudine	3056-17-5	224.2	-0.81*	
Streptomycin	57-92-1	581.6		
Sulfadiazine	68-35-9	250.3		
Sulfadimethoxine	121-11-2	310.33	1.63 (4.0)*	5.9a
Sulfamerazine	127-79-7	264.30	0.14*	7.1a
Sulfamethazine	57-68-1	278.33	0.28 (4.0)*	2.36c, 7.38a
Sulfisoxazole	127-69-5	267.30	1:01*	5.0a
Sumatriptan	103628-46-2	295.40	-1.17	
Tetracycline	60-54-8	444.45	-1.05 (5.3) -1.44 (7.5)	3.3a, 7.7a, 9.7c
Theophylline	58-55-9	180.19	-0.02*	0.7c, 8.8a
Thiopental	76-75-5		2.85*	7.45a
Thioridazine	50-52-2	370.56	5.90	9.5c
Thiotepa	52-24-4	189.2	0.53*	
Tolazoline	59-98-3	160.21	2.65	10.3c
Tramadol	27203-92-5	263.39	2.63(7.4)*	
Verapamil	52-53-9	454.59	3.79 (9.0)*	8.75c
Warfarin	81-81-2	308.35	2.70*	5.0a
Zalcitabine	7481-89-2	211.2	-1.30*	

[4] Bruggeman *et al.* (1984).

When pH is equal to the pK_a, unionised and ionised moieties exist in equal proportion. The degree of ionisation of acidic drugs increases with increasing pH and acidic drugs with lower pK_a values will tend to ionise more easily. In the case of basic drugs, the converse is true and the degree of ionisation decreases with increasing pH and drugs with higher pK_a values will tend to ionise more easily. This phenomenon is important as the degree of ionisation will influence lipid solubility. In turn, lipid solubility is relevant to important fate and effects processes such as adsorption, volatilisation, ecotoxicity and bio-accumulation. In general, neutral compounds will be more lipid soluble and consequently more highly adsorbed to organic solids, more toxic and bioconcentrated to a greater degree than their ionised counterparts. It is notable that most medicinal compounds are either weak acids or weak bases. One evident shortcoming in the consideration of the effective environmental exposure of pharamaceuticals is the general lack of experience in dealing with the fate of ionisable compounds. This is particularly the case for wastewater treatment processes. Whereas the predominant influence upon the adsorption of neutral compounds to organic solids during wastewater treatment is hydrophobicity, interactions with ionised compounds are less well understood. Under circumstances where a significant proportion of a compound is present as the ionised moiety, actual adsorption to organic solids (K_{oc}) will deviate from that predicted for the neutral moiety from hydrophobicity alone (i.e., log K_{OW}) and adsorption will therefore also be a function of pKa and ambient pH. Following a consideration of the pK_a values, it is likely that the degree of removal via adsorption during wastewater treatment of many pharmaceuticals will deviate from that predicted from hydrophobicity alone. Biomass/bacterial matter within wastewater treatment plants is negatively charged. As such anionically charged compounds (i.e., acids) will tend to be repulsed by negatively charged bacteria. The corollary is that cationically charged compounds (i.e., bases) will tend to be attracted by the biomass. For reference, the typical operational pH of activated sludge treatment plants is ~6.5. Under such conditions, acidic compounds with pK_a values <6.5 will be present predominantly as the anionic moiety, whereas

basic compounds with pK_a >6.5 will be present as the cationic moiety. One of the few empirical studies to deal with the effects of pH upon the sludge/water distribution coefficient (k_d) of an ionisable compound is Ziegenfuss and Hannah (1994). Their study demonstrated how adsorption of 2,4,5-Trichlorophenol (pK_a 7.4) to activated sludge solids was positively related to the proportion of unionised (i.e., neutral compound). Overall, this is an area that requires further research.

BIODEGRADATION DATA

Richardson & Bowron (1985) selected 25 human pharmaceutical compounds for biodegradation studies "on the basis of high quantity in use, potential for being noxious or because on reviewing the literature the drug seemed to survive sewage treatment". The results from these studies are presented below (Table 2). Compounds highlighted as 'Non-biodegradable' failed to comply with test criteria (Ready Biodegradation Test) and are not necessary totally recalcitrant and persistent. However, no information on by-products or metabolites was reported. In the case of the anti-microbials, test concentration may have influenced the outcome of the testing. The relevance or applicability of the tests *per se* may also be questioned as they are typically conducted at relatively high concentrations (mg/l range). This is greatly in excess of likely real-world concentrations. In addition to biodegradation, other degradation/removal process may apply (i.e., photodegradation, adsorption, hydrolysis, volatilisation etc.). For example, the photodegradation half-life of aqueous Tetracycline is reported to range from 88 - 130 hours (Peterson *et al.*, 1993). Likewise, the photodegradation half-life of Diclofenac is reported as <1 hour in a Swiss lake (Buser *et al.*, 1998a).

Henschel *et al.* (1997) reports on the ready biodegradability (OECD 301F) of Salicylic Acid, Paracetamol, Clofibrinic Acid and Methotrexate. Only Salicylic Acid yielded a categorical positive result. Paracetamol was borderline and both Clofibrinic acid and Methotrxate failed.

Table 2. - Biodegradation results for 25 selected human pharmaceutical compounds (from Richardson & Bowron, 1985).

Drug	Category	Biodegradability[5]
Amitriptyline	Anti-depressant	'Non-biodegradable'
Ampicillin	Anti-bacterial	'Inherently biodegradable'
Aspirin	Analgesic; Anti-pyretic; Anti-inflammatory	'Readily biodegradable'
Caffeine	CNS stimulant	'Readily biodegradable'
Chlorhexidine	Topical anti-bacterial; Disinfectant	'Non-biodegradable'
Clofibrate	Anti-hyperlipoproteinemic	'Non-biodegradable'
Codeine Phosphate	Narcotic analgesic; Anti-tussive	'Non-biodegradable'
Dextropropoxyphene	Narcotic analgesic	'Non-biodegradable'
Ephedrine	Bronchodilator; Decongestant	'RB + acclimation'
Erythromycin	Anti-bacterial	'Non-biodegradable'
Ibuprofen	Anti-inflammatory	'Inherently biodegradable'
Menthol	Topical anti-pruritic	'Readily biodegradable'
Meprobamate	Anxiolytic	'Non-biodegradable'
Methyldopa	Anti-hypertensive	'Non-biodegradable'
Metronidazole	Anti-protozoal	'Non-biodegradable'
Naproxen[6]	Anti-inflammatory; Analgesic; Anti-pyretic	'Non-biodegradable'

5 'RB + acclimation' denotes readily biodegradable after acclimation.

Nicotinamide	Vasodilator	'Readily biodegradable'
Paracetamol[7]	Analgesic; Anti-pyretic	'RB + acclimation'
Phenylpropanolamine	Decongestant: Anorexic	'RB + acclimation'
Sulphamethoxazole	Anti-bacterial; Anti-pneumocystis	'Non-biodegradable'
Sulphasalazine	Treatment of ulcerative co-litis & Crohn's disease	'Non-biodegradable'
Tetracycline	Anti-amoebic; Anti-bacterial; Anti-ricketettsial	'Non-biodegradable'
Theobromine	Diuretic; Bronchodilator; Cardiotonic	'RB + acclimation'
Theophylline	Bronchodilator	'Readily biodegradable'
Tolbutamide	Anti-diabetic	'Non-biodegradable'

Another study has demonstrated that both Iopromide and Ethinyl Oestradiol are non-readily biodegradable (Schweinfurth *et al.*, 1996). Ingerslev *et al.* (1998) similarly reports that both Oxytetracycline and Metronidazole are non-readily biodegradable. Kümmerer *et al.* (1996) reports limited biodegradation of Ifosfamide and Cyclophosphamide in the Closed Bottle Test (CBT - OECD 301D). Similarly, the limited biodegradation of Cefotiam, Ciprofloxacin, Ifosfamide, Meropenem, Metronidazole, Ofloxacin, Penicillin and Sulphamethoxazole is described by Kümmerer *et al.* (1997), Al-Ahmad *et al.* (1999) and Kümmerer *et al.* (2000). The biodegradation profile of 12 sulfonamides is reported by Ingersleev & Halling-Sorensen (2000). None were degraded in screening tests conducted at concentrations below microbial

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[6] Yan *et al.* (1995) reports a marked degree of biodegradation of Naproxen.

[7] Wotzka *et al.* (1994) reports a BOD_5/COD ratio of 73.68% for Paracetamol following adaption (BOD_5/COD >50% = 'Readily Biodegradable').

inhibitory thresholds. Al-Ahmad *et al.* (2001) report that *Vinca* alkaloid antineoplastics are not readily biodegrable in the CBT test. The antineoplastics Mitoxantron and Treosulfane are similarly reported as non-readily biodegradable by Al-Ahmad (1997). The general lack of biodegradation of iodinated x-ray contrast media is highlighted in Steger-Hartmann *et al.* (1998; 1999). Ready biodegradation test methodologies (i.e., OECD 301A-F) are given in OECD (1992).

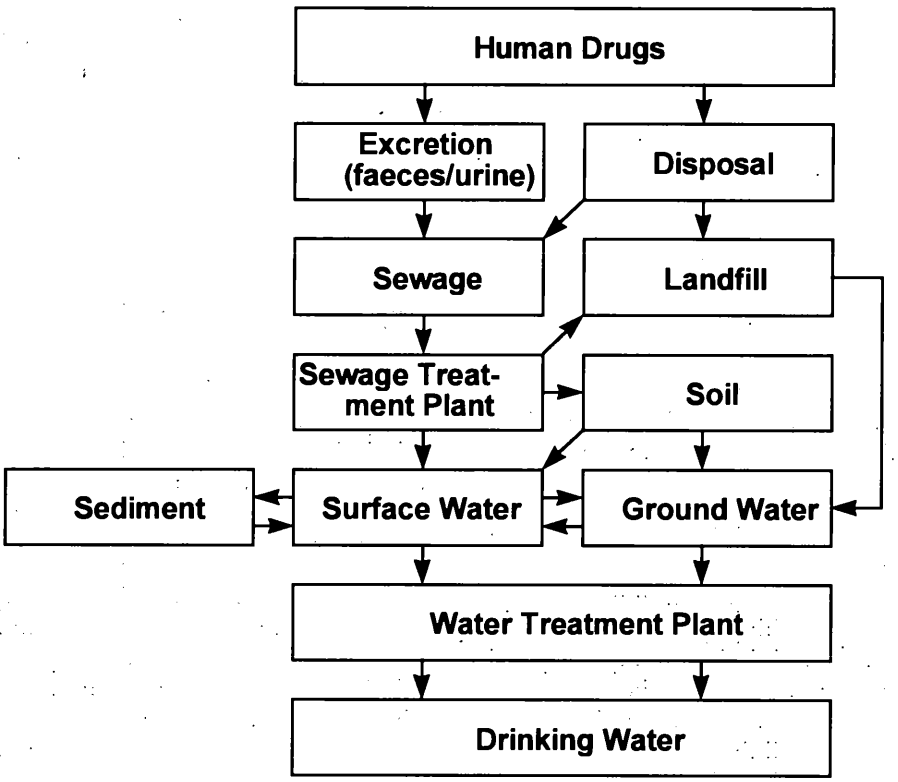
ENVIRONMENTAL CONCENTRATIONS

Possible routes of environmental exposure to human pharmaceuticals are depicted in Figure 1. A certain proportion of drugs will typically be excreted from the body via urine or faeces following administration as the parent material or as a metabolite. This will enter the sewage before arriving at the sewage treatment plant (STP). A proportion of unused drugs may also be present in sewage following disposal to drain. During sewage treatment, a certain proportion of any compound will be typically be removed by biodegradation and/or adsorption to sewage sludge solids. Adsorbed compounds will typically be applied to land during sludge application, incinerated or landfilled. Landfill is also a sink for medicines diposed of via the solid waste stream. Discharges from sewage treatment plants will contribute to surface water and sediment loadings. Groundwater is potentially contaminated via landfill leachate or exchange with sludge amended soils or surface waters. Surface waters will also be potentially subject to contamination from surface run-off. Potable water is extracted from surface waters or groundwater.

In considering the fate of pharmaceuticals, Richardson and Bowron (1985) paid particular attention to anti-neoplastic agents, immunosuppresants, compounds with morphinian substructure, oral contraceptives, Penicillins and Aspirin. Environmental concentrations of such pharmaceuticals that were observed or summarised by Richardson and Bowron (1985) are

presented below (see Table 3) together with observations from other subsequent studies. Most notable amongst these is Ternes (1998) who conducted a comprehensive survey of the occurrence 32 drugs in German sewage effluent discharges and rivers. In total, over 60 compounds are listed.

Figure 1. - Routes of environmental exposure for human pharmaceuticals.



Overall, most observations from the various studies were in the ng/l range with maximal concentrations for some compounds in the low µg/l range. The highest concentrations were unsurprisingly associated with untreated influent (i.e., raw sewage) from hospitals. Observations of Caffeine were attributed to beverages, rather than drugs (Richardson & Bowron, 1985). The presence of Aspirin in effluent has at least partly been attributed to microbial degradation of naphthalene oils from oil spillages, rather than pharmaceuticals (Richardson & Bowron 1985). It is notable that Paracetamol, the most frequently consumed pharmaceutical, was not recorded as being detected in surface waters by Richardson & Bowron (1985) or Ternes (1998). Rogers *et al.* (1986) report the detection of Ibuprofen, Naproxen and Clofi-

bric Acid in primary treated sewage effluent without details of concentrations. The extremely high values (i.e., µg/l range) for Ethinyl Oestradiol reported by Tabak *et al.* (1981) contrast markedly with other reported observations (i.e., ng/l range) and should therefore be treated with some caution.

Table 3. - Observed environmental concentrations (ng/l) of human pharmaceuticals in raw sewage, sewage effluent, river water and potable water.

Drug	Sewage Influent	Sewage Effluent	River Waters	Potable & Ground Waters	Reference
Aspirin	-	~ 1,000	-	-	Richardson & Bowron (1985)
Aspirin	-	<50 - 1510	-	-	Stumpf *et al.* (1996)
Aspirin	-	(i) median 220 (ii) maximum 1500	(i) median <20 (ii) maximum 340	-	Ternes (1998)
Betaxolol	-	(i) median 57 (ii) maximum 190	(i) median <10 (ii) maximum 28	-	Ternes (1998)

Bezafibrate	-	(i) median 2,200 (ii) maximum 4,600	(i) median 350 (ii) maximum 3,100	-	Ternes (1998)
Bisoprolol	-	(i) median 57 (ii) maximum 370	(i) median <10 (ii) maximum 2,900	-	Ternes (1998)
Bleomycin	-	(i) range 11 - 19 (ii) mean 15.8	(i) range <5 - 17 (ii) mean 8.5	(i) range 5 - 13 (ii) mean 8.7	Aherne *et al.* (1990)
Caffeine	-	~ 1,000	-	> 1,000	Richardson & Bowron (1985)
Caffeine	-	16 - 292	-	-	Rogers *et al.* (1986)
Caffeine	-	2,000 - 33,000	-	-	Paxéus (1996)
Carazolol	-	(i) median <25 (ii) maximum 120	(i) median <10 (ii) maximum 110	-	Ternes (1998)
Carbamazepine	-	(i) median 2,100 (ii) maximum 6,300	(i) median 250 (ii) maximum 1,100	-	Ternes (1998)

Chloramphenicol	-	(i) median <20 (ii) maximum 560	(i) median <20 (ii) maximum 60	<20	Hirsch *et al.* (1999)
Chlorotetracycline	-	<50	<50	<50	Hirsch *et al.* (1999)
Ciprofloxacin	8,000 - 87,000 (hospital)	-	-	-	Hartmann *et al.* (1998)
Ciprofloxacin	range 5,000 - 90,000 (hospital)	primary 250 - 370 final 70 - 80	-	-	Alder *et al.* (1998)
Clarithromycin	-	240	(i) median <20 (ii) maximum 260	<20	Hirsch *et al.* (1999)
Clenbuterol	-	(i) median <50 (ii) maximum 80	<10	-	Ternes (1998)
Clofibric Acid	-	-	~ 40	-	Richardson & Bowron (1985)
Clofibric Acid	-	Detected	-	-	Rogers *et al.* (1986)

Clofibric Acid	-	-	<222	10 - 165	Stan *et al.* (1994)
Clofibrinic Acid	-	-	<1 - 100	-	Kalbfus (1995b)
Clofibric Acid	-	-	<0.5 - 1,750 (Berlin)	-	Heberer (1995)
Clofibric Acid	-	-	<0.5 - 220 (Europe)	-	Heberer (1995)
Clofibric Acid	-	<50 - 1560	<5 - 180	-	Stumpf *et al.* (1996)
Clofibric Acid	-	-	-	70 - 7,300	Heberer *et al.* (1997)
Clofibric Acid	-	-	Swiss Lakes <1 - 9 North Sea ~0.5 - 7.8	-	Buser *et al.* (1998b)
Clofibrate	-	<100	<30	-	Ternes (1998)
Clofibric Acid	-	(i) median 360 (ii) maximum 1,600	(i) median 66 (ii) maximum 550	-	Ternes (1998)
Cloxacillin	-	<20	<20	<20	Hirsch *et al.* (1999)

Cyclophosphamide	146 (hospital)	-	-	-	Steger-Hartmann *et al.* (1996)
Cyclophosphamide	-	(i) median <10 (ii) maximum 20	<10	-	Ternes (1998)
Dextropropoxyphene	-	-	~ 1,000	-	Richardson & Bowron (1985)
Diazepam	-	< 1,000	~ 10	~ 10	Waggott (1981)
Diazepam	-	(i) median <30 (ii) maximum 50	<30	-	Ternes (1998)
Dichlorfenac	-	<2,000	15 - 304 (Rhine) 38 - 489	-	Stumpf *et al.* (1996)
Dichlorfenac	-	-	-	nd - 380	Heberer *et al.* (1997)
Diclofenac	-	-	range <1 - 12 (lakes) range 11 - 310 (rivers)	-	Buser *et al.* (1998a)

Diclofenac	-	(i) median 810 (ii) maximum 2,100	(i) median 150 (ii) maximum 1,200	-	Ternes (1998)
Dicloxacillin	-	<20	<20	<20	Hirsch *et al.* (1999)
Diethylstilbestrol	-	-	-	(i) range 0 - 0.8 (ii) mean 0.11 - 0.24	Rurainski *et al.* (1977)
Dimethylamino-phenazone	-	(i) median <100 (ii) maximum 1,000	(i) median <30 (ii) maximum 340	-	Ternes (1998)
Doxcycline	-	<50	<50	<50	Hirsch *et al.* (1999)
Erythromycin	-	-	~ 1,000	-	Watts *et al.* (1983)
Erythromycin	-	(i) median 2500 (ii) maximum 6,000	(i) median 150 (ii) maximum 1,700	<20	Hirsch *et al.* (1999)

Ethinyl Oestradiol	-	-	-	(i) range 0 - 22.5 (ii) mean 0.69 - 3.18	Rurainski *et al.* (1977)
Ethinyl Oestradiol	(i) mean 1210 (ii) range 500 - 2250 (sic)	(i) mean 810 (ii) range 250 - 1780 (sic)	-	-	Tabak *et al.* (1981)
Ethinyl Oestradiol	-	-	<5	< 5	Aherne, English & Marks (1985)
Ethinyl Oestradiol	-	<1 - 7	2 - 15	<1 - 4	Aherne & Briggs (1989)
Ethinyl Oestradiol	-	0.5 - 1.1	-	-	FWR (1992)
Ethinyl Oestradiol	-	0.3 - 0.5	<0.2	<0.2	Kalbfus (1995)
Ethinyl Oestradiol	-	(i) over half <0.2 (ii) where detected 0.2 - 7.0	-	-	Desbrow *et al.* (1996)
Ethinyl Oestradiol	~10	-	-	-	Belfroid *et al.* (1998)

Ethinyl Oestradiol	-	0.76	-	-	Snyder *et al.* (1998)
Ethinyl Oestradiol	-	-	-	<0.4	James *et al.* (1998)
Ethinyl Oestradiol	-	(i) median 1 [D] & 9 [Can] (ii) maximum 15 [D] & 42 [Can]	<0.5 [D]	-	Ternes *et al.* (1999)
Etofibrate	-	<100	<30	-	Ternes (1998)
Fenofibrate	-	-	-	nd - 45	Heberer *et al.* (1997)
Fenofibrate	-	<50	<10	-	Ternes (1998)
Fenofibric Acid	-	(i) median 380 (ii) maximum 1,200	(i) median 45 (ii) maximum 280	-	Ternes (1998)
Fenoprofen	-	<50	<10	-	Ternes (1998)
Fenoterol	-	(i) median <50 (ii) maximum 60	(i) median <10 (ii) maximum 61	-	Ternes (1998)

Gembibrozil	-	(i) median 400 (ii) maximum 1,500	(i) median 52 (ii) maximum 510	-	Ternes (1998)
Ibuprofen	-	Detected	-	-	Rogers *et al.* (1986)
Ibuprofen	-	<12,000	<5 - 41 (Rhine) 17 - 139	-	Stumpf *et al.* (1996)
Ibuprofen	-	-	-	nd - 200	Heberer *et al.* (1997)
Ibuprofen	-	(i) median 370 (ii) maximum 3,400	(i) median 70 (ii) maximum 530	-	Ternes (1998)
Ibuprofen	990 - 3,300	2 - 81	<0.2 - 7.8	-	Buser *et al.* (1999)
Ifosamide	24 (hospital)	-	-	-	Steger-Hartmann *et al.* (1996)
Ifosamide	Median 109 (hospital) Median 6.2 - 8.5 (communal)	Median 6.5 - 9.3 (communal)	-	-	Kümmerer *et al.* (1997)

Ifosamide	-	(i) median <10 (ii) maximum 2,900	<10	-	Ternes (1998)
Indometacine	-	(i) median 270 (ii) maximum 600	(i) median 40 (ii) maximum 200	-	Ternes (1998)
Ketoprofen	-	(i) median 200 (ii) maximum 280	(i) median <10 (ii) maximum 120	-	Ternes (1998)
Meclofenamic Acid	-	<50	<10	-	Ternes (1998)
Methaqualone	~ 1,000 (hospital)	-	-	-	Richardson & Bowron (1985)
Methicillin	-	<20	<20	<20	Hirsch *et al.* (1999)
Methotrexate	~ 1,000 (oncology clinic)	-	< 6.25	< 6.25	Aherne, English & Marks (1985)
Metoprolol	-	(i) median 730 (ii) maximum 2,200	(i) median 45 (ii) maximum 2,200	-	Ternes (1998)

"Morphinian Sub-structure"	-	-	< 1,000	-	Richardson & Bowron (1985)
Nadolol	-	(i) median 25 (ii) maximum 60	<10	-	Ternes (1998)
Nafcillinn	-	<20	<20	<20	Hirsch *et al.* (1999)
Naproxen	-	Detected	-	-	Rogers *et al.* (1986)
Naproxen	-	(i) median 300 (ii) maximum 520	(i) median 70 (ii) maximum 390	-	Ternes (1998)
Norethisterone	-	-	-	< 10	Aherne, English & Marks (1985)
Norethisterone	-	8 - 20	<2 - 17	<2 - <10	Aherne & Briggs (1989)
"Oral Contraceptives"	< 100	-	< 200	-	Aherne, English & Marks (1985)
Oxacillin	-	<20	<20	<20	Hirsch *et al.* (1999)

Oxytetracycline	-	<50	<50	<50	Hirsch *et al.* (1999)
Paracetamol/Acetaminophen	-	(i) median <500 (ii) maximum 6,000	<150	-	Ternes (1998)
"Penicilloyl Groups"	-	-	< 25	< 10	Richardson & Bowron (1985)
Penicillin G	-	<20	<20	<20	Hirsch *et al.* (1999)
Penicillin V	-	<20	<20	<20	Hirsch *et al.* (1999)
Phenazone	-	-	-	<10 - 1,250	Heberer *et al.* (1997)
Phenazone	-	(i) median 160 (ii) maximum 410	(i) median 24 (ii) maximum 950	-	Ternes (1998)
Propranolol	-	(i) median 170 (ii) maximum 290	(i) median 12 (ii) maximum 590	-	Ternes (1998)
Propyphenazone	-	-	-	nd - 1,465	Heberer *et al.* (1997)

Roxithromycin	-	(i) median 680 (ii) maximum 1,000	(i) median <20 (ii) maximum 560	<20	Hirsch *et al.* (1999)
Salbutamol	-	(i) median <50 (ii) maximum 170	(i) median <10 (ii) maximum 35	-	Ternes (1998)
Sulfamethazine	-	<20	<20	(i) median <20 (ii) maximum 160	Hirsch *et al.* (1999)
Sulfamethoxazole	-	-	~ 1,000	-	Watts *et al.* (1983)
Sulfamethoxazole	-	(i) median 400 (ii) maximum 2,000	(i) median 30 (ii) maximum 480	(i) median <20 (ii) maximum 470	Hirsch *et al.* (1999)
Terbutalin	-	(i) median <50 (ii) maximum 120	<10	-	Ternes (1998)
Tetracycline	-	-	~ 1,000	-	Watts *et al.* (1983)

Tetracycline	-	<50	<50	<50	Hirsch *et al.* (1999)
Theophylline	-	-	~ 1,000	-	Watts *et al.* (1983)
Timolol	-	(i) median <25 (ii) maximum 70	(i) median <10 (ii) maximum 10	-	Ternes (1998)
Tolfenamic Acid	-	<50	<10	-	Ternes (1998)
Trimethoprim	-	(i) median 320 (ii) maximum 660	(i) median <20 (ii) maximum 200	<20	Hirsch *et al.* (1999)

WASTEWATER TREATMENT REMOVAL

Several publications deal with the fate of human pharmaceuticals during wastewater treatment. This is an important consideration as most sewage influent containing drug residues excreted via faeces and/or urine will be subject to wastewater treatment. The degree of removal during wastewater treatment is a major determinand of aquatic exposure. Reported observations are presented in Table 4 (AS denotes activated sludge and TF trickling filter).

Table 4. - Reported removal of pharmaceutical residues during wastewater treatment.

Compound	WWTP Removal (%)	WWTP Type	Reference
Acetylsalicyclic Acid	81	AS	Ternes (1998)

Bezafibrate	83	AS	Ternes (1998)
Bezafibrate	27 50	TF AS	Stumpf *et al.* (1999)
Carbamazepine	7	AS	Ternes (1998)
Clofibric Acid	51	AS	Ternes (1998)
Clofibric Acid	15 34	TF AS	Stumpf *et al.* (1999)
Diclofenac	69	AS	Ternes (1998)
Diclofenac	9 75	TF AS	Stumpf *et al.* (1999)
Dimethylaminophenazoen	38	AS	Ternes (1998)
Ethinyl Oestradiol	64 78 0	TF AS AS	Ternes *et al.* (1999)
Fenofibric Acid	64	AS	Ternes (1998)
Fenofibric Acid	6 45	TF AS	Stumpf *et al.* (1999)
Gemfibrozil	69	AS	Ternes (1998)
Gemfibrozil	16 46	TF AS	Stumpf *et al.* (1999)
Ibuprofen	22 75	TF AS	Stumpf *et al.* (1999)
Ibuprofen	90	AS	Ternes (1998)
Ibuprofen	96 - 99.9	AS	Buser *et al.* (1999)
Indometacine	75	AS	Ternes (1998)

Indometacine	71 83	TF AS	Stumpf *et al.* (1999)
Ketoprofen	48 69	TF AS	Stumpf *et al.* (1999)
Metroprolol	83	AS	Ternes (1998)
Naproxen	66	AS	Ternes (1998)
Naproxen	15 78	TF AS	Stumpf *et al.* (1999)
Phenazone	33	AS	Ternes (1998)
Propranolol	96	AS	Ternes (1998)

DISCUSSION

The presence of pharmaceuticals in the environment may arise from various sources such as manufacture, use or disposal. Within the current context, the key route of entry of human pharmaceuticals into the environment is a consequence of use by patients within the community or within hospitals or clinics etc. Patients may excrete pharmaceuticals (or their metabolites) in urine or faeces or dispose of unused medicines via the sewage system. Emissions from pharmaceutical production facilities are typically highly controlled and are not thought to be responsible for the diffuse distribution of the wide range of compounds observed in the environment as highlighted here. The environmental exposure pathways (including potential transformation and depletion/removal mechanisms) of drugs in general have been reviewed by Velagaleti (1997).

Once within the sewage system drug residues will typically be subject to wastewater treatment. Likely fate during waste water treatment will be dependent upon a variety of parameters relating to physico-chemistry (i.e., K_{ow}, pK_a, water solubility) and degradation via biologi-

cal (i.e., biodegradation) or chemical (i.e., hydrolysis) pathways. Type of treatment will also be a major consideration. For example, Stumpf *et al.* (1999) demonstrate the higher efficiency of activated sludge treatment relative to trickling filter for all compounds considered. Differences in fate during wastewater treatment can be illustrated by reference to different compounds. Compounds which are easily biodegraded show near complete removal (via mineralisation) during wastewater treatment e.g., 98% for Paracetamol (Ternes 1998). Other relatively hydrophilic compounds (which may include conjugates) which are also more resistant to biodegradation will pass through the wastewater treatment plant so that overall removal is more limited e.g., 34% for Clofibric acid (Stumpf *et al.*, 1999). The possibility for microbially mediated deconjugation of conjugates during wastewater treatment should also be considered as a factor potentially influencing ultimate environmental release. Other more hydrophobic substances may exhibit relatively high removal during treatment due to absorption to solids rather than via biodegradation e.g., Penicillins (Halling-Sørensen *et al.*, 1998).

The large number of compounds that have been detected in the environment is amply illustrated in Table 3. Chief amongst these are analgesics, lipid regulators, antibiotics, steroids and anti-neoplastics. Concentrations vary according to the medium analysed. As expected, higher concentrations are associated with sewage influents and effluents than surface waters. This is well illustrated by the observations of Ternes (1998). Most observations in surface waters are in the ng/l range, although a few compounds have been measured in the µg/l range e.g., Clofibric Acid (Herberer *et al.*, 1997). A major determinand of drug residue concentration in surface waters will be the extent of dilution of the sewage effluent following discharge from the treatment plant. Ternes (1998) clearly demonstrates how drug concentration is a function of river/stream size. There are relatively few reports of pharmaceuticals in water supplies or drinking water (e.g., Heberer *et al.*, 1997). Others have typically failed to detect pharmaceuticals in such water supplies (e.g., Hirsch *et al.*, 1999). An exten-

sive discussion of fate and observed environmental concentrations *per se* will not be undertaken here. This has adequately been dealt with elsewhere (e.g., EA, 2000). The main thrust of this study is the benchmarking of predicted and observed concentrations against available ecotoxicity data. The data in this chapter have been collated to assist in this purpose.

CONCLUSIONS

The increasing number of observations of pharmaceutical compounds in sewage, surface waters and drinking water supplies mean that the environmental fate of pharmaceuticals cannot and should not be ignored. These observations are associated with use of medicinal products by patients rather than emissions from manufacturing facilities. They imply exposure of aquatic biota and potentially indirect human exposure. This necessitates risk assessment. Risk assessment requires effects data against which to benchmark exposure. These points are addressed in the subsequent chapters.

REFERENCES

Aherne GW, Briggs R (1989) The relevance of the presence of certain synthetic steroids in the aquatic environment. *J. Pharm. Pharmacol.* 41: 735-736.

Aherne GW, English J, Marks V (1985) The role of immunoassay in the analysis of micro-contaminants in water samples. *Ecotoxicology and Environmental Safety* 9: 79-83.

Aherne GW, Hardcastle A, Nield AH (1990) Cytotoxic drugs and the aquatic environment: estimation of bleomycin in river and water samples. *J. Pharm. Pharmacol.* 42: 741-742.

Al-Ahmad A, Kümmerer K, Schön (1997) Biodegradation and toxicity of antineoplastics Mitoxantron Hydrochloride and Tresulfane in the Closed Bottle Test (OECD 301 D). *Bull. Environ. Contam. Toxicol.* 58: 704-711.

Al-Ahmad A, Daschner FD, Kümmerer K (1999) Biodegradability of Cefotiam, Ciprofloxacin, Meropenem, Penicillin G and Sulfamethoxazole and inhibition of waste water bacteria. *Arch. Environ. Contam. Toxicol.* 37: 158-163.

Al-Ahmad A, Kümmerer K (2001) Biodegradation of the antineoplastic Vindesine, Vincristine and Vinblastine and their toxicity against bacteria in the aquatic environment. *Cancer Detection and Prevention* 25(1): 102-107.

Alder AC, Gole, E, Hartmann A, Ibric S, Suter MJF (1998) Occurrence of the fluoroquinolone antibiotic Ciprofloxacin in hospital and municipal wastewaters. SETAC 19th Annual Meeting 15-19th November 1998 Charlotte, NC [Abstract 329].

Belfroid AC, Murk AJ, De Voogt P, Cofino WP, Schafer AJ (1998) Fate of estrogenic compounds in waste water treatment. SETAC Europe 8th Annual Meeting 14-18 April 1998 Bordeaux [Abstract 3H/013].

Bowman WC, Rand MJ (1980) *Textbook of Pharmacology* (2nd edition). Blackwell Scientific Publications: Oxford.

Bruggeman WA, Weber-Fung D, Opperhuizen A, Van Der Steen J, Wijbenga A, Hutzinger O (1984) Absorption and retention of polydimethylsiloxanes in Fish: Preliminary experiments. *Toxicological & Environmental Chemistry* 7: 287-296.

Buser H-R, Müller MD, Theobald N (1998b) Occurrence of the pharmaceutical drug clofibric acid and the herbicide mecoprop in various Swiss lakes and the North Sea. *Environ. Sci. Technol.* 32: 188-192.

Buser H-R, Poiger T, Müller MD (1998a) Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: rapid photodegradation in a lake. *Environ. Sci. Technol.* 32: 3449-3546.

Buser H-R, Poiger T, Müller MD (1999) Occurrence and environmental behaviour of the chiral pharmaceutical drug Ibuprofen in surface waters and in wastewater. *Environ. Sci. Technol.* 33: 2529-2535.

Clements RG, Nabholz JV, Johnson DE, Zeeman MG (1993) The use of quantitative structure-activity relationships (QSARs) as screening tools in environmental assessment. In: "*Environmental Toxicology and Risk Assessment: 2nd Volume, ASTM STP 1216*" (Eds. JW Gorsuch, FJ Dwyer, CG Ingersoll, TW La Point). 555-570. American Society for Testing and Materials: Philadelphia (PA, USA).

Desbrow C, Routledge E, Sheahan D, Waldock M, Sumpter JP (1996) *The identification of oestrogenic substances in sewage treatment effluents.* Report of the Environment Agency: Bristol, U.K.

Dollery CT [Ed.] (1991) *Therapeutic Drugs - Volume 1 & 2.* Churchill Livingstone: Edinburgh.

EA (2000) *Review of Human Pharmaceuticals in the Environment.* R & D Technical Report P390 (Ayscough NJ, Fawell J, Franklin G & Young W). Research Contractor WRc_NSF Ltd. Environment Agency.

FWR (1992) *Effects of Trace Organics on Fish [October 1992 FR/D 0008].* Foundation for Water Research: Marlow (Bucks.), UK.

Geyer HJ, Scheunert I, Brüggemann R, Steinberg C, Korte F, Kettrup A (1991) QSAR for organic chemical bioconcentration in *Daphnia*, algae and mussels. *The Science of the Total Environment* 109/110: 387-394.

Halling-Sørenson, B, Nors Nielsen, S., Lanzky, P.F., Ingerslev, Holten Lützhøft & H.C. & Jørgensen, S.E. (1998). Occurrence, fate and effects of pharmaceutical substances in the environment - A review. *Chemosphere* 36(2): 357-393.

Hansch C, Leo A, Hoekman D (1995) *Exploring QSAR - Hydrophobic, Electronic and Steric Constants.* ACS Professional Reference Book, American Chemical Society: Washington DC (USA).

Hartmann A, Alder AC, Koller T, Widmer RM (1998) Identification of Fluoroquinolone antibiotics as the main source of *umuC gen*otoxicity in native hospital wastewater. *Environmental Toxicology and Chemistry* 17(3), 377-382.

Heberer, T. (1995) Identifizierung und quanttifizerung von pestiidrückständen und umweltkontaminanten in grund- und oberflächenwässern mittels kapillar-gas chromatographic-massenspektoskopic. Wissenschaft und Technik Verlag, Berlin 376S. Quoted in Halling-Sørensen et al. (1998).

Heberer T, Dunnbier U, Reilich CH, Stan H-J (1997) Detection of drugs and drug metabolites in groundwater samples of a drinking water treatment plant. *Fresenius Envir. Bull.* 6: 438-443.

Henschel KP, Wenzel A, Diederich M, Fliedner A (1997) Environmental Hazard Assessment of Pharmaceuticals. *Regulatory Toxicology & Pharmacology* 25: 220-225.

Hirsch R, Ternes T, Haberer K, Kratz K-L (1999) Occurrence of antibiotics in the aquatic environment. *Sci. Tot. Env.* 225: 109-118.

Hoekman D (1997) http://clogp.edu/medchem/chem/clogp/drugs.html [Internet Site].

Ingerslev F & Halling-Sørensen B (2000) Biodegradability properties of sulfonamides in activated sludge. *Environmental Toxicology & Chemistry* 19(10): 2467-2473.

Ingerslev F, Holten-Lützhøft H-C, Halling-Sørensen B (1998) Hazard identification and biodegradation of three high volume veterinary drugs. SETAC-Europe 8th Annual Meeting 14-18th April 1998 Bordeaux [Abstract 4I/005].

James HA, Fielding M, Franklin O, Williams D, Lunt D (1998) *Steroid Concentrations in Treated Sewage Effluents and Water Courses - Implications for Water Supplies.* Report Ref. No. 98/TX/01/1. UK Water Industry Research Limited: London.

Kaiser KLE, Esterby SR (1991) Regression and cluster analysis of the acute toxicity of 267 chemicals to six species of biota and the octanol/water partition coefficient. *The Science of the Total Environment* 109/110: 499-514.

Kalbfus W (1995) Belastung bayerisches Gewässer durch synthetische Östrogene. Vortag bei der 50. Fachtagung des Bayerisches Landesamt für Wasserwirtschaft: Stoffe mit endokriner Wirkung im Wasser (Abstract). [Effects in Bavarian watercourses through synthetic oestrogens. Presentation at the 50th Seminar of the Bavarian Association for Waters Supply: Substances with endocrine effects in water (Abstract)].

Kalbfus W (1995b) Belastung bayerisches Gewässer durch Lipidsenker. Vortag bei der 50. Fachtagung des Bayerisches Landesamt für Wasserwirtschaft: Stoffe mit endokriner Wirkung im Wasser (Abstract). [Effects in Bavarian watercourses through anti-hyperlipoproteinemics . Presentation at the 50th Seminar of the Bavarian Association for Waters Supply: Substances with endocrine effects in water (Abstract)].

Karickhoff SW (1981) Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. *Chemosphere* 10: 833-846.

Könemann H (1981) Quantitative-structure activity relationships in fish toxicity studies. *Toxicology* 19: 209-211.

Kümmerer K, Steger-Hartmann T, Baranyai A, Bürhaus I (1996) Evaluation of the biological degradation of the antineoplastics cyclophosphamide and ifosfamide with the Closed Bottle Test (OECD 301D). *Zbl. Hyg.* 198: 215-225.

Kümmerer K, Steger-Hartmann T, Meyer M (1997) Biodegradability of the anti-tumour agent ifosfamide and its occurrence in hospital effluents and communal sewage. *Water Research* 31(11): 2705-2710.

Kümmerer K, Al-Ahmad A, Mersch-Sundermann (2000) Biodegradability of some antibiotics, elimination of the genotoxicity and affectation of wastewater bacteria in simple tests. *Chemosphere* 40(7): 701-710.

Mackay D (1982) Correlation of bioconcentration factors. *Environ. Sci. Technol.* 16: 274–276.

OECD (1992) OECD Guidelines for the Testing of Chemicals/Section 3: Degradation and Accumulation Test Np. 301: Ready Biodegradation. OECD, Paris.

Newton DW, Kluza RB (1978) pK_a values of medicinal compounds in pharmacy practice. *Drug Intell. Clin. Pharm.* 12: 546-554.

Paxéus N (1995) Organic pollutants in the effluents of large wastewater treatment plants in Sweden. *Water Research* 30(5): 1115-1122.

Peterson SM, Batley GE, Scammell MS (1993) Tetracycline in antifouling paints. *Marine Pollution Bulletin* 26(2): 96-100.

Raymond GG, Born JL (1986) An updated pK_a listing of medicinal compounds. *Drug. Intell. Clin. Pharm.* 20: 683-686.

Richardson ML, Bowron JM (1985) The fate of pharmaceutical chemicals in the aquatic environment. *J. Pharm. Pharmacol.* 37: 1-12.

Rogers IH, Birtwell IK, Kruzynski GM (1986) Organic extractables in municipal wastewater Vancouver, British Columbia. *Water Poll. Res. J. Canada* 21(2): 187-204.

Rurainski R, Theiss H-J, Zimmermann W (1977) Uber das vorkommen von näturlichen und synthetischen oestrogenen im trinkwasser [Concerning the occurrence of natural and synthetic oestrogens in drinking water]. *Gwf-Wasser/Abwasser* 118(6).

Schweinfurth H, Länge R, Schneider PW (1996) Environmental risk assessment in the pharmaceutical industry. Presentation at the "*3rd Eurolab Symposium - Testing and Analysis for Industrial Competitiveness and Sustainability*" Berlin, 5 - 7th June, 1996.

Snyder SA, Keith TL, Snyder EM, Kannan K, Geisy JP (1998) Methods for determining ethinylestradiol concentrations in water samples. SETAC 19th Annual Meeting 15-19th November 1998 Charlotte, NC [Abstract PMP0023].

Stan H-J, Heberer T, Linkerhägner M (1994) Vorkommen von clofibrinsäure im aquatischen System - Führt die therapeutische Anwendung zu einer Belastung von Oberfllächen-, Grund- und Trinkwasser? - [Occurrence of clofibric acid in the aquatic system - Is the use in human medical care the source of the contamination of surface, ground and drinking water?]. *Vom Wasser* 83: 57-68.

Steger-Hartmann T, Kümmerer K, Schecker J (1996) Trace analysis of the antineoplastics ifosamide and cyclophosphamide in sewage water by two-step solid phase extraction and gas chromatography-mass spectrometry. *Journal of Chromatography A* 726: 179-184.

Steger-Hartmann T, Länge R, Schweinfurth H (1998) Environmental fate and ecotoxicological assessment of iodinated x-ray contrast media. *Vom Wasser* 91: 185-194.

Steger-Hartmann T, Länge R, Schweinfurth H (1999) Environmental risk assessment for the widely used iodinated x-ray contrast agent Iopromide (Ultravist). *Ecotoxicology and Environmental Safety (Environmental Research, Section B)* 42: 274–281.

Stumpf M, Ternes TA, Wilken R-D, Rodrigues SV, Baumann W (1999) Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. *Sci. Tot. Env.* 225: 135-141.

Stumpf M, Ternes TA, Haberer K, Seel, P, Baumann W (1996) Nachweis von arzneimittelrückständen in kläranlagen und fliessgewässern. Vom *Wasser* 86: 291-303.

Tabak HH, Bloomhuff RN, Bunch RL (1981) Steroid hormones as pollutants II. Studies on the persistence and stability of natural urinary and synthetic ovulation-inhibiting hormones in untreated and treated wastewaters. In: "*Developments in Industrial Microbiology* (22)", 497-519, Proc. 37, General Meeting of the Soc. Industr. Microbiol., Aug. 1980, Flagstaff, Garamomd/Pridemark Press: Baltimore.

Ternes TA (1998) Occurrence of drugs in German sewage treatment plants and rivers. *Water Research* 32(11): 3245-3260.

Ternes TA, Stumpf M, Mueller J, Haberer K, Wilken RD, Servos M (1999) Behaviour and occurrence of oestrogens in municipal sewage treatment plants - I. Investigations in Germany, Canada and Brazil. *Sci. Tot. Env.* 225: 81-90.

Veith GD, Kosian P (1983) Estimating bioconcentration potential from octanol/water partition coefficients. In: "*PCBs in the Great Lakes*" (Eds. D. Mackay, R., Paterson, S. Eisenreich & M. Simmons), Chapter 15. Ann Arbor Science, Ann Arbor (MI, USA).

Velagaleti R (1997) Behaviour of pharmaceuticals drugs (human and animal health) in the environment. *Drug Information Journal* 31: 715-722.

Waggott A (1981) Trace organic substances in the River Lee - In: "*Chemistry in Water Reuse*" [Ed. Cooper, W.J.], pp 55-99. Ann. Arbor Publishers Inc.; Ann Arbor (MT, USA). Quoted in Richardson & Bowron (1985).

Watts CD, Crathorne B, Fielding M, Steel CP (1983) Identification of non-volatile organics in water using field desorption mass spectrometry and high performance liquid chromatography. In: "*Analysis of Organic Micropollutants in Water*" [Eds. G. Angeletti and A. Bjørseth], pp 120-131. D.D. Reidel Publishing Company; Dordrech.

Wotzka J, Pfitzner S, Giest B (1994) Biochemische Abbaubarkeit ausgewählter organischer Verbindungen; Teil 2 [Biochemical degradability of selected organic compounds; Part 2]. *Dtsch. Gewasserkunde Mitt. (DGM)* 38(1/2): 10-17.

Yan Z, Marple L, Samples M, Velalaleti RR (1995) Aquatic biodegradation of Naproxen Sodium. SETAC (Society for Environmental Toxicology and Chemistry) Second World Congress [Vancouver BC, November 1995] - Abstract PW241.

Ziegenfuss PS, Hannah RE (1994) Adsorption of ionizable chemicals to activated sludge. Preprinted extended abstract presented before the Division of Environmental Chemistry, at the 208th American Chemical Society National Meeting [Washington DC, August 21 - 25 1994] Volume 34(2): 541-543.

CHAPTER 3

ECOTOXICITY DATA

This chapter has been published in "*Pharmaceuticals in the Environment – Sources, Fate, Effects and Risks*" (Ed. K. Kümmerer) under the title "*A Data Based Perspective on the Environmental Risk Assessment of Human Pharmaceuticals I – Collation of Available Ecotoxicity Data*" pp 175-201 (Springer, 2001).

INTRODUCTION

There is a growing literature relating to observations of human pharmaceuticals in the environment (see review by Halling-Sørenson *et al.*, 1998). Discussions about the environmental consequences of the presence of such compounds have taken place in the general absence of a systematic analysis of the potential risk. This can partly be attributed to the lack of public domain information relating to the ecotoxicity of pharmaceuticals. The lack of such an analysis means that to date, decisions concerning Environmental Risk Assessment criteria and/or regulatory thresholds have been somewhat arbitrary or based upon inappropriate groups of industrial chemicals such as pesticides. This study attempts to address that deficiency and collates examples of data relating to the ecotoxicity of existing human pharmaceuticals. The intention is to provide perspective that will prove useful during the further development of assessment criteria. The database may also prove useful in the context of the risk assessment of individual substances.

METHODS

A review of available acute ecotoxicity data for macro-invertebrates, fish and algae was conducted. Results of studies from searches of the published scientific literature were supplemented with details of studies from the grey-literature or regulatory submissions secured via contacts with colleagues from industry and academia. In collating the data, attempts were made to ensure that the original sources of data were consulted. In many cases it was not always possible to establish if concentrations at the reported endpoints relate to nominal or measured concentrations. Inclusion of data does not imply endorsement - in terms of quality - of the study in question. A list of test of species is given in the Appendix (page 108).

RESULTS

Acute Ecotoxicity Data

The available acute ecotoxicity database for macro-invertebrates, fish and algae is presented in Table 1. There are over 360 endpoints for over 100 human pharmaceuticals.

Table 1. - Acute ecotoxicity data for human pharmaceuticals.

Compound	Category[8]	Value (mg/l)	Endpoint/ Duration[9]	Species	Reference
Acarbose	Anti-diabetic	>1000	EC_{50}	Unspecified fish	FDA-CDER (1996)
Acarbose	Anti-diabetic	>1000	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Acriflavine	Anti-infective	5	96h LC_{50}	*Morone saxatilis* (larvae)	Hughes (1973)
Acriflavine	Anti-infective	30.0	48h LC_{50}	*Morone saxatilis* (fingerling)	Hughes (1973)
Acriflavine	Anti-infective	28.0	72h LC_{50}	*Morone saxatilis* (fingerling)	Hughes (1973)
Acriflavine	Anti-infective	27.5	96h LC_{50}	*Morone saxatilis* (fingerling)	Hughes (1973)

8 Therapeutic category is as detailed in the Merck Index (Budavari, 1989).

9 LC_{50} values relate to lethality in all organisms. EC_{50} values in Daphnia typically relate to immobilisation. In the case of algae, EC_{50} values relate to effects upon growth (i.e., biomass or cell number). US FDA test guidelines include 4.01 algal assay, 4.08 *Daphnia* acute toxicity (48h), 4.09 Daphnia chronic testing, 4.10 *Hyalella azteca* acute toxicity and 4.11 freshwater fish acute toxicity.

Acriflavine	Anti-infective	30.1	24h LC_{50}	*Oncorhynchus mykiss*	Wilford (1966)
Acriflavine	Anti-infective	19.9	48h LC_{50}	*Oncorhynchus mykiss*	Wilford (1966)
Acriflavine	Anti-infective	37.5	24h LC_{50}	*Salvelinus namaycush*	Wilford (1966)
Acriflavine	Anti-infective	28.0	48h LC_{50}	*Salvelinus namaycush*	Wilford (1966)
Acriflavine	Anti-infective	40.0	24h LC_{50}	*Salmo trutta*	Wilford (1966)
Acriflavine	Anti-infective	27.0	48h LC_{50}	*Salmo trutta*	Wilford (1966)
Acriflavine	Anti-infective	43.5	24h LC_{50}	*Ictalurus punctatus*	Wilford (1966)
Acriflavine	Anti-infective	33.2	48h LC_{50}	*Ictalurus punctatus*	Wilford (1966)
Acriflavine	Anti-infective	48.0	24h LC_{50}	*Salvelinus fontinalis*	Wilford (1966)
Acriflavine	Anti-infective	14.8	48h LC_{50}	*Salvelinus fontinalis*	Wilford (1966)
Acriflavine	Anti-infective	18.0	24h LC_{50}	*Lepomis macrochirus*	Wilford (1966)
Acriflavine	Anti-infective	13.5	48h LC_{50}	*Lepomis macrochirus*	Wilford (1966)
Alendronate Sodium	Metabolic Bone Disease	1450	LC_{50}	*Pimephales promelas*	FDA-CDER (1996)
Alendronate Sodium	Metabolic Bone Disease	>1000	LC_{50}	*Oncorhynchus mykiss*	FDA-CDER (1996)

Alendronate Sodium	Metabolic Bone Disease	22	LC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Alendronate Sodium	Metabolic Bone Disease	>0.5	MIC	Green Algae	FDA-CDER (1996)
Aminosidine	Anti-bacterial; Anti-amebic	2,220	48h EC_{50}	*Artemia*	Migliore *et al.* (1997)
Aminosidine	Anti-bacterial; Anti-amebic	847	72h EC_{50}	*Artemia*	Migliore *et al.* (1997)
Aminosidine Sulphate (Neomycin E)	Anti-bacterial; Anti-amebic	1,055	24h LC_{50}	*D. magna*	Di Delupis *et al.* (1992)
Aminosidine Sulphate (Neomycin E)	Anti-bacterial; Anti-amebic	503	48h LC_{50}	*D. magna*	Di Delupis *et al.* (1992)
Amitriptyline	Anti-depressant	1.2	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Amitriptyline	Anti-depressant	36.9	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Amitriptyline	Anti-depressant	0.78	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Amitriptyline	Anti-depressant	5.55	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Amitriptyline	Anti-depressant	0.80	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Amobarbital	Sedative; Hypnotic	85.4	96h EC_{50}	*Pimephales promelas*	Russom *et al.* (1997)

Amopyroquin Dihydrochloride	Anti-malarial	47.0	24h LC_{50}	*Oncorhynchus mykiss*	Willford (1966)
Amopyroquin Dihydrochloride	Anti-malarial	35.3	48h LC_{50}	*Oncorhynchus mykiss*	Willford (1966)
Amopyroquin Dihydrochloride	Anti-malarial	15.5	24h LC_{50}	*Salvelinus namaycush*	Willford (1966)
Amopyroquin Dihydrochloride	Anti-malarial	14.0	48h LC_{50}	*Salvelinus namaycush*	Willford (1966)
Amopyroquin Dihydrochloride	Anti-malarial	42.0	24h LC_{50}	*Salmo trutta*	Willford (1966)
Amopyroquin Dihydrochloride	Anti-malarial	36.0	48h LC_{50}	*Salmo trutta*	Willford (1966)
Amopyroquin Dihydrochloride	Anti-malarial	19.8	24h LC_{50}	*Ictalurus punctatus*	Willford (1966)
Amopyroquin Dihydrochloride	Anti-malarial	12.5	48h LC_{50}	*Ictalurus punctatus*	Willford (1966)
Amopyroquin Dihydrochloride	Anti-malarial	52.0	24h LC_{50}	*Salvelinus fontinalis*	Willford (1966)
Amopyroquin Dihydrochloride	Anti-malarial	40.0	48h LC_{50}	*Salvelinus fontinalis*	Willford (1966)
Amopyroquin Dihydrochloride	Anti-malarial	33.0	24h LC_{50}	*Lepomis macrochirus*	Willford (1966)
Amopyroquin Dihydrochloride	Anti-malarial	18.5	48h LC_{50}	*Lepomis macrochirus*	Willford (1966)
Amphetamine Sulphate	CNS stimulant; Anorexic	28.8	96h EC_{50}	*Pimephales promelas*	Russom *et al.* (1997)

Amphetamine Sulphate	CNS stimulant; Anorexic	60	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Amphetamine Sulphate	CNS stimulant; Anorexic	1515	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Amphetamine Sulphate	CNS stimulant; Anorexic	55	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Amphetamine Sulphate	CNS stimulant; Anorexic	270	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Amphetamine Sulphate	CNS stimulant; Anorexic	4.90	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Aprotinin	Enzyme Inhibitor (protease)	>1,000	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Aspirin	Analgesic; Anti-pyretic; Anti-inflammatory	1468	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Aspirin	Analgesic; Anti-pyretic; Anti-inflammatory	382	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Aspirin	Analgesic; Anti-pyretic; Anti-inflammatory	178	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)

Aspirin	Analgesic; Anti-pyretic; Anti-inflammatory	168	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Aspirin	Analgesic; Anti-pyretic; Anti-inflammatory	141	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Atropine Sulphate	Anti-cholinergic; Mydriatic	258	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Atropine Sulphate	Anti-cholinergic; Mydriatic	15,773	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Atropine Sulphate	Anti-cholinergic; Mydriatic	661	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Atropine Sulphate	Anti-cholinergic; Mydriatic	356	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Atropine Sulphate	Anti-cholinergic; Mydriatic	334	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Azithromycin	Anti-bacterial	>120	LC_{50}	Unspecified amphipod	FDA-CDER (1996)
Azithromycin	Anti-bacterial	120	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)

Bacitracin	Anti-bacterial	34.1	24h EC_{50}	*Artemia salina* (nauplii)	Migliore *et al.* (1997)
Bacitracin	Anti-bacterial	21.8	48h EC_{50}	*Artemia salina* (nauplii)	Migliore *et al.* (1997)
Bacitracin	Anti-bacterial	34.1	24h LC_{50}	*Artemia salina* (nauplii)	Brambilla *et al.* (1994)
Bacitracin	Anti-bacterial	21.8	48h LC_{50}	*Artemia salina* (nauplii)	Brambilla *et al.* (1994)
Bacitracin	Anti-bacterial	126.4	24h LC_{50}	*D.magna*	Brambilla *et al.* (1994)
Bacitracin	Anti-bacterial	30.5	48h LC_{50}	*D.magna*	Brambilla *et al.* (1994)
Bacitracin	Anti-bacterial	126.4	24h LC_{50}	*D.magna*	Di Delupis *et al.* (1992)
Bacitracin	Anti-bacterial	30.5	48h LC_{50}	*D.magna*	Di Delupis *et al.* (1992)
Bicalutamide	Non-steroidal Anti-androgen	>5	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Bicalutamide	Non-steroidal Anti-androgen	>1	EC_{50}	Unspecified green algae	FDA-CDER (1996)
Bicalutamide	Non-steroidal Anti-androgen	>1	EC_{50}	Unspecified blue-green algae	FDA-CDER (1996)
Budesonide	Anti-inflammatory	20	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Budesonide	Anti-inflammatory	>19	LC_{50}	Unspecified fish	FDA-CDER (1996)

Caffeine	CNS stimulant	151	96h EC_{50}	*Pimephales promelas*	Russom *et al.* (1997)
Caffeine	CNS stimulant	684	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Caffeine	CNS stimulant	3,457	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Caffeine	CNS stimulant	410	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Caffeine	CNS stimulant	160	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Caffeine	CNS stimulant	4,661	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Carvedilol	Anti-hypertensive; Anti-anginal	>3	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Carvedilol	Anti-hypertensive; Anti-anginal	1	LC_{50}	Unspecified fish	FDA-CDER (1996)
Cefprozil	Anti-bacterial	>642	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Ceftibuten	Anti-bacterial	>600	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Ceftibuten	Anti-bacterial	>520	LC_{50}	Amphipod	FDA-CDER (1996)
Cetirizine HCl	Anti-histaminic	330	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Chloramine T	Anti-bacterial	23.6	24h LC_{50}	*Penaeus setiferus*	Johnson (1976)
Chloramine T	Anti-bacterial	22	96h LC_{50}	*Rasbora heteromorpha*	Tooby *et al.* (1975)

Chloramphenicol	Anti-bacterial; Anti-rickettsial	543	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Chloramphenicol	Anti-bacterial; Anti-rickettsial	2,042	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Chloramphenicol	Anti-bacterial; Anti-rickettsial	305	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Chloramphenicol	Anti-bacterial; Anti-rickettsial	1,086	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Chloramphenicol	Anti-bacterial; Anti-rickettsial	2,074	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Chloroquine Phosphate	Anti-malarial; Anti-amebic; Anti-rheumatic	50	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Chloroquine Phosphate	Anti-malarial; Anti-amebic; Anti-rheumatic	2,043	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Chloroquine Phosphate	Anti-malarial; Anti-amebic; Anti-rheumatic	11.7	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)

Chloroquine Phosphate	Anti-malarial; Anti-amebic; Anti-rheumatic	43.5	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Chloroquine Phosphate	Anti-malarial; Anti-amebic; Anti-rheumatic	4.39	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Cimetidine	Anti-ulcerative	740	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Cimetidine	Anti-ulcerative	>1000	LC_{50}	*Lepomis macrochirus*	FDA-CDER (1996)
Cisapride	Peristaltic Stimulant	>1000	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Cisapride	Peristaltic Stimulant	>1000	LC_{50}	*Lepomis macrochirus*	FDA-CDER (1996)
Cladribine	Anti-neoplastic	233	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Clofibrate	Anti-hyperlipoprotein-emic	28.2	24h EC_{50}	*D. magna*	Köpf (1995)
Clofibrate	Anti-hyperlipoprotein-emic	12.0	EC_{50}	Unspecified algae	Köpf (1995)
Clofibrinic Acid	Anti-hyperlipoprotein-emic	106	EC_{50}	*D. magna*	Henschel *et al.* (1997)

Clofibrinic Acid	Anti-hyperlipoprotein-emic	86.0	48h EC_{50}	*Brachydanio rerio* (embryos)	Henschel *et al.* (1997)
Clofibrinic Acid	Anti-hyperlipoprotein-emic	89	72h EC_{50}	*Scenedesmus subspicatus*	Henschel *et al.* (1997)
Cyclosporine	Immunosuppressant	>100	LC_{50}	*Oncorhynchus mykiss*	FDA-CDER (1996)
Cyclosporine	Immunosuppressant	20	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Dextropropoxyphene HCl	Narcotic analgesic	14.6	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Dextropropoxyphene HCl	Narcotic analgesic	308	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Dextropropoxyphene HCl	Narcotic analgesic	7.6	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Dextropropoxyphene HCl	Narcotic analgesic	19	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Dextropropoxyphene HCl	Narcotic analgesic	4.2	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Diazepam	Anxiolytic; Muscle Relaxant	65.4	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Diazepam	Anxiolytic; Muscle Relaxant	103	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)

Diazepam	Anxiolytic; Muscle Relax-ant	14.1	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Diazepam	Anxiolytic; Muscle Relax-ant	>10,000	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Diazepam	Anxiolytic; Muscle Relax-ant	4.3	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Didanosine	Anti-(retro)viral	>1,020	EC_{50}	*D. magna*	FDA-CDER (1996)
Diethylstilbestrol	Estrogen	4.0	LC_{50}	*D. magna*	Coats *et al.* (1976)
Diethylstilbestrol	Estrogen	>10	LC_{50}	*Physa* spp.	Coats *et al.* (1976)
Diethylstilbestrol	Estrogen	>1	48h LC_{50}	*Gambusia affinis*	Coats *et al.* (1976)
Diethylstilbestrol	Estrogen	1.09	48h LC_{50}	*D. magna*	Zou & Fingerman (1997)
Diethylstilbestrol	Estrogen	1.2	48h LC_{50}	*D. magna*	Baldwin *et al.* (1995)
Diethylstilbestrol	Estrogen	316	14d LC_{50}	*Pimephales promelas*	Panter *et al.* (1999)
Digoxin	Cardio-tonic	24	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Dirithromycin	Anti-bacterial	>2,880	LC_{50}	*Oncorhynchus mykiss*	FDA-CDER (1996)
Dirithromycin	Anti-bacterial	>48	EC_{50}	*D. magna*	FDA-CDER (1996)

Dorzolamide HCl	Carbonic anhydrase inhibitor, treatment of glaucoma	>1,000	LC_{50}	*Pimephales promelas*	FDA-CDER (1996)
Dorzolamide HCl	Carbonic anhydrase inhibitor, treatment of glaucoma	699	EC_{50}	*D. magna*	FDA-CDER (1996)
Erythromycin	Anti-bacterial	388	24h LC_{50}	*D.magna*	Di Delupis *et al.* (1992)
Erythromycin	Anti-bacterial	211	48h LC_{50}	*D.magna*	Di Delupis *et al.* (1992)
Erythromycin Phosphate	Anti-bacterial	818	24h LC_{50}	*Salvelinus namaycush*	Marking *et al.* (1988)
Erythromycin Phosphate	Anti-bacterial	410	96h LC_{50}	*Salvelinus namaycush*	Marking *et al.* (1988)
Erythromycin Thiocyanate	Anti-bacterial	>80	48h LC_{50}	*Oncorhynchus mykiss, Salmo trutta, Salvelinus fontinalis, Ictalurus punctatus, Lepomis macrochirus & Salvelinus namaycush*	Wilford (1966)
Ethinyl Oestradiol	Estrogen	5.7	24h EC_{50}	*D. magna*	Köpf (1995)
Ethinyl Oestradiol	Estrogen	0.84	EC_{50}	Unspecified algae	Köpf (1995)

Ethinyl Oestradiol	Estrogen	6.4	48h EC_{50}	*D. magna*	Schweinfurth *et al.* (1996)
Ethinyl Oestradiol	Estrogen	1.6	96h EC_{50}	*Oncorhynchus mykiss*	Schweinfurth *et al.* (1996)
Etidronic Acid	Metabolic Bone Disease	200	96h LC_{50}	*Oncorhynchus mykiss*	Gledhill & Feijtel (1992)
Etidronic Acid	Metabolic Bone Disease	868	96h LC_{50}	*Lepomis macrochirus*	Gledhill & Feijtel (1992)
Etidronic Acid	Metabolic Bone Disease	695	48h LC_{50}	*Ictalurus punctatus*	Gledhill & Feijtel (1992)
Etidronic Acid	Metabolic Bone Disease	3.0	96h EC_{50}	Unspecified algae	Gledhill & Feijtel (1992)
Etidronic Acid	Metabolic Bone Disease	527	48h EC_{50}	*D. magna*	Gledhill & Feijtel (1992)
Famciclovir	Anti-viral	>986	LC_{50}	*Lepomis macrochirus*	FDA-CDER (1996)
Famciclovir	Anti-viral	820	EC_{50}	*D. magna*	FDA-CDER (1996)
Famotidine	Anti-ulcerative	>680	LC_{50}	*Pimephales promelas*	FDA-CDER (1996)
Famotidine	Anti-ulcerative	398	EC_{50}	*D. magna*	FDA-CDER (1996)
Finasteride	Treatment of benign prostatic hypertrophy	21	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)

Finasteride	Treatment of benign prostatic hypertrophy	20	LC_{50}	*Oncorhynchus mykiss*	FDA-CDER (1996)
Flumazenil	Benzodiazepine Antagonist	>500	EC_{50}	*D. magna*	FDA-CDER (1996)
Flumequine	Anti-bacterial	476.8	24h EC_{50}	*Artemia salina* (nauplii)	Migliore *et al.* (1997)
Flumequine	Anti-bacterial	307.7	48h EC_{50}	*Artemia salina* (nauplii)	Migliore *et al.* (1997)
Flumequine	Anti-bacterial	96.4	72h EC_{50}	*Artemia salina* (nauplii)	Migliore *et al.* (1997)
Flumequine	Anti-bacterial	477	24h LC_{50}	*Artemia salina* (nauplii)	Brambilla *et al.* (1994)
Flumequine	Anti-bacterial	308	48h LC_{50}	*Artemia salina* (nauplii)	Brambilla *et al.* (1994)
Flumequine	Anti-bacterial	96.4	72h LC_{50}	*Artemia salina* (nauplii)	Brambilla *et al.* (1994)
Flutamide	Androgen	>1,000	14d LC_{50}	*Pimephales promelas*	Panter *et al.* (1999)
Fluticasone Propionate	Corticosteroid anti-asthmatic	0.55	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Fluoxetine HCl	Anti-depressant	0.94	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Fluoxetine HCl	Anti-depressant	2.0	LC_{50}	*Oncorhynchus mykiss*	FDA-CDER (1996)

Fluoxetine HCl	Anti-depressant	0.031	EC_{50}	Unspecified green algae	FDA-CDER (1996)
Fluoxetine	Anti-depressant	1.55	4h LOEC	*Sphaerium* spp.	Fong *et al.* (1998)
Fluvoxamine Male-ate	Anti-Depressant	63	MIC	Unspecified algae	FDA-CDER (1996)
Fluvoxamine	Anti-Depressant	0.003	4h LOEC	*Sphaerium striati-num*	Fong *et al.* (1998)
Gabapentin	Anti-epileptic adjunctive	>1,100	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Ibuprofen	Analgesic; anti-inflammatory	7.1	96h EC_{50}	*Skeletonema co-statum*	Knoll/BASF (1995)
Ibuprofen	Analgesic; anti-inflammatory	9.06	48h EC_{50}	*D. magna*	Knoll/BASF (1995)
Ibuprofen	Analgesic; anti-inflammatory	173	96h LC_{50}	*Lepomis macrochi-rus*	Knoll/BASF (1995)
Iopromide	Diagnostic Aid (radiopaque medium)	>962	LC_{50}	*Oncorhynchus my-kiss*	FDA-CDER (1996)
Iopromide	Diagnostic Aid (radiopaque medium)	>973	LC_{50}	*Lepomis macrochi-rus*	FDA-CDER (1996)
Iopromide	Diagnostic Aid (radiopaque medium)	137	MIC	Unspecified green algae	FDA-CDER (1996)

Iopromide	Diagnostic Aid (radiopaque medium)	>1,016	EC_{50}	*Daphnia*	FDA-CDER (1996)
Iopromide	Diagnostic Aid (radiopaque medium)	>10,000	24h EC_{50}	*D. magna*	Schweinfurth *et al.* (1996a)
Iopromide	Diagnostic Aid (radiopaque medium)	>10,000	48h EC_{50}	Unspecified fish	Schweinfurth *et al.* (1996a)
Isoniazid	Anti-bacterial	85	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Isoniazid	Anti-bacterial	322	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Isoniazid	Anti-bacterial	24.4	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Isoniazid	Anti-bacterial	125.5	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Isoniazid	Anti-bacterial	3,045	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Ketorolac Tromethamine	Analgesic; Anti-inflammatory	1480	96h LC_{50}	*Lepomis macrochirus*	Anon (1993)
Lansoprazole	Proton pump inhibitor (Anti-ulcerative)	>22	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)

Lansoprazole	Proton pump inhibitor (Anti-ulcerative)	18	LC_{50}	*Oncorhynchus mykiss*	FDA-CDER (1996)
Lincomys(c)in	Anti-bacterial	283.1	72h EC_{50}	*Artemia*	Migliore *et al.* (1997)
Lincomys(c)in	Anti-bacterial	379.39	72h LC_{50}	*D.magna*	Di Delupis *et al.* (1992)
Lithium Sulphate	Anti-depressant	197	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Lithium Sulphate	Anti-depressant	4,318	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Lithium Sulphate	Anti-depressant	112	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Lithium Sulphate	Anti-depressant	33.1	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Lithium Sulphate	Anti-depressant	712	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Lomefloxacin	Anti-bacterial	130	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Lomefloxacin	Anti-bacterial	170	LC_{50}	*Oncorhynchus mykiss*	FDA-CDER (1996)
Lomefloxacin	Anti-bacterial	2.4	EC_{50}	Unspecified green algae	FDA-CDER (1996)
Loracarbef	Anti-infective	>963	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Losartan K	Anti-hypertensive	331	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)

Losartan K	Anti-hypertensive	>929	LC_{50}	*Oncorhynchus mykiss*	FDA-CDER (1996)
Losartan K	Anti-hypertensive	>1000	LC_{50}	*Pimephales promelas*	FDA-CDER (1996)
Losartan K	Anti-hypertensive	245	MIC	Unspecified green alage	FDA-CDER (1996)
Losartan K	Anti-hypertensive	949	MIC	Unspecified blue-green alage	FDA-CDER (1996)
Merthiolate (Thimerosal)	Anti-infective	60.5	24h LC_{50}	*Oncorhynchus mykiss*	Wilford (1966)
Merthiolate (Thimerosal)	Anti-infective	21.2	48h LC_{50}	*Oncorhynchus mykiss*	Wilford (1966)
Merthiolate (Thimerosal)	Anti-infective	13.0	24h LC_{50}	*Salvelinus namaycush*	Wilford (1966)
Merthiolate (Thimerosal)	Anti-infective	2.13	48h LC_{50}	*Salvelinus namaycush*	Wilford (1966)
Merthiolate (Thimerosal)	Anti-infective	110	24h LC_{50}	*Salmo trutta*	Wilford (1966)
Merthiolate (Thimerosal)	Anti-infective	54.0	48h LC_{50}	*Salmo trutta*	Wilford (1966)
Merthiolate (Thimerosal)	Anti-infective	7.50	24h LC_{50}	*Ictalurus punctatus*	Wilford (1966)
Merthiolate (Thimerosal)	Anti-infective	5.65	48h LC_{50}	*Ictalurus punctatus*	Wilford (1966)
Merthiolate (Thimerosal)	Anti-infective	89.5	24h LC_{50}	*Salvelinus fontinalis*	Wilford (1966)

Merthiolate (Thimerosal)	Anti-infective	74.5	48h LC_{50}	*Salvelinus fontinalis*	Wilford (1966)
Merthiolate (Thimerosal)	Anti-infective	110	24h LC_{50}	*Lepomis macrochirus*	Wilford (1966)
Merthiolate (Thimerosal)	Anti-infective	64.5	48h LC_{50}	*Lepomis macrochirus*	Wilford (1966)
Metformin HCl	Anti-diabetic	>982	LC_{50}	*Lepomis macrochirus*	FDA-CDER (1996)
Metformin HCl	Anti-diabetic	130	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Methotrexate	Anti-neoplastic; Anti-rheumatic	>1,000	EC_{50}	*D. magna*	Henschel *et al.* (1997)
Methotrexate	Anti-neoplastic; Anti-rheumatic	85.0	48h EC_{50}	*Brachydanio rerio* (embryos)	Henschel *et al.* (1997)
Methotrexate	Anti-neoplastic; Anti-rheumatic	260	72h EC_{50}	*Scenedesmus subspicatus*	Henschel *et al.* (1997)
Metronidazole	Anti-protozoal	>100	72h EC_{50}	*Acartia tonsa*	Lanzky & Halling-Sørenson (1997)
Metronidazole	Anti-protozoal	>500	96h EC_{50}	*Brachydanio rerio*	Lanzky & Halling-Sørenson (1997)
Metronidazole	Anti-protozoal	39.1	72h EC_{50}	*Selenastrum capricornutum*	Lanzky & Halling-Sørenson (1997)
Metronidazole	Anti-protozoal	12.5	72h EC_{50}	*Chlorella* spp.	Lanzky & Halling-Sørenson (1997)

Metronidazole	Anti-protozoal	>100	48h LC_{50}	*Oncorhynchus mykiss, Salmo trutta, Salvelinus fontinalis, Ictalurus punctatus, Lepomis macrochirus & Salvelinus namaycush*	Wilford (1966)
Midazolam	Anesthetic (intravenous)	0.2	EC_{50}	*D. magna*	FDA-CDER (1996)
Milrinone Lactate	Cardiotonic	414	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Moexipril HCl (pro-drug)	Anti-hypertensive	800	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Moexiprilat (active metabolite)	Anti-hypertensive	>1000	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Naproxen Sodium	Anti-inflammatory; Analgesic; Anti-pyretic	140	24h EC_{50}	*D. magna*	Rodriguez *et al.* (1992)
Naproxen Sodium	Anti-inflammatory; Analgesic; Anti-pyretic	383	96h LC_{50}	*Hyalella azteca*	Rodriguez *et al.* (1992)

Naproxen Sodium	Anti-inflammatory; Analgesic; Anti-pyretic	560	96h LC_{50}	*Lepomis macrochirus*	Rodriguez *et al.* (1992)
Naproxen Sodium	Anti-inflammatory; Analgesic; Anti-pyretic	690	96h LC_{50}	*Oncorhynchus mykiss*	Rodriguez *et al.* (1992)
Nefazodone HCl	Anti-depressant	7	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Nicotine Sulphate	Cholinergic agonist	13.8	96h EC_{50}	*Pimephales promelas*	Russom *et al.* (1997)
Nicotine	Cholinergic agonist	3.0	EC_{50}	*D. magna*	FDA-CDER (1996)
Nicotine	Cholinergic agonist	7.0	LC_{50}	*Oncorhynchus mykiss*	FDA-CDER (1996)
Nicotine	Cholinergic agonist	20.0	LC_{50}	*Pimephales promelas*	FDA-CDER (1996)
Nicotine	Cholinergic agonist	4.0	LC_{50}	*Lepomis macrochirus*	FDA-CDER (1996)
Nicotine	Cholinergic agonist	13	LC_{50}	"*Goldfish*"	FDA-CDER (1996)
Nisoldipine	Anti-hypertensive; Anti-anginal	33	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)

Nisoldipine	Anti-hypertensive; Anti-anginal	3	EC_{50}	Unspecified fish	FDA-CDER (1996)
Nitrofurazone	Topical Anti-infective	1.45	EC_{50}	*Selenastrum capricornutum*	Macrì & Sbardella (1984)
Nitrofurazone	Topical Anti-infective	28.7	LC_{50}	*D. magna*	Macrì & Sbardella (1984)
Nitrofurazone	Topical Anti-infective	10	96h LC_{50}	*Morone saxatilis* (larvae)	Hughes (1973)
Nitrofurazone	Topical Anti-infective	>5	24h LC_{50}	*Penaeus setiferus*	Johnson (1976)
Omeprazole	Anti-ulcerative	88	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Ondansetron HCl	Anti-emetic	28	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Orphenadrine HCl (Mephenamin)	Relaxant; Anti-histaminic	8.9	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Orphenadrine HCl (Mephenamin)	Relaxant; Anti-histaminic	45	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Orphenadrine HCl (Mephenamin)	Relaxant; Anti-histaminic	4.3	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Orphenadrine HCl (Mephenamin)	Relaxant; Anti-histaminic	10.6	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Orphenadrine HCl (Mephenamin)	Relaxant; Anti-histaminic	5.4	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Oxytetracycline	Anti-bacterial	>5	24h LC_{50}	*Penaeus setiferus*	Johnson (1976)

Oxytetracycline HCl	Anti-bacterial	62.5	24/48/72 /96h LC_{50}	*Morone saxatilis* (larvae)	Hughes (1973)
Oxytetracycline HCl	Anti-bacterial	150	24h LC_{50}	*Morone saxatilis* (fingerling)	Hughes (1973)
Oxytetracycline HCl	Anti-bacterial	125	48h LC_{50}	*Morone saxatilis* (fingerling)	Hughes (1973)
Oxytetracycline HCl	Anti-bacterial	100	72h LC_{50}	*Morone saxatilis* (fingerling)	Hughes (1973)
Oxytetracycline HCl	Anti-bacterial	75	96h LC_{50}	*Morone saxatilis* (fingerling)	Hughes (1973)
Oxytetracycline HCl	Anti-bacterial	<200	24/96h LC_{50}	*Salvelinus namay-cush*	Marking *et al.* (1988)
Oxytetracycline	Anti-bacterial	0.231	EC_{50}	*Microcystis aerugi-nosa*	Holten-Lützhøft *et al.* (1998)
Oxytetracycline	Anti-bacterial	5.0	EC_{50}	*Selenastrum capri-cornutum*	Holten-Lützhøft *et al.* (1998)
Oxytetracycline	Anti-bacterial	1.7	EC_{50}	*Rhodomonas*	Holten-Lützhøft *et al.* (1998)
Paclitaxel	Anti-neoplastic	>0.74	LC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Paracetamol/ Acetaminophen	Analgesic; Anti-pyretic	577	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Paracetamol/ Acetaminophen	Analgesic; Anti-pyretic	29.6	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)

Paracetamol/ Acetaminophen	Analgesic; Anti-pyretic	55.5	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Paracetamol/ Acetaminophen	Analgesic; Anti-pyretic	5,306	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Paracetamol/ Acetaminophen	Analgesic; Anti-pyretic	13	24h EC_{50}	*D. magna*	Kühn *et al.* (1989)
Paracetamol/ Acetaminophen	Analgesic; Anti-pyretic	9.2	48h EC_{50}	*D. magna*	Kühn *et al.* (1989)
Paracetamol/ Acetaminophen	Analgesic; Anti-pyretic	293	24 EC_{50}	*D. magna*	Henschel *et al.* (1997)
Paracetamol/ Acetaminophen	Analgesic; Anti-pyretic	50.0	48 EC_{50}	*D. magna*	Henschel *et al.* (1997)
Paracetamol/ Acetaminophen	Analgesic; Anti-pyretic	378	48h EC_{50}	*Brachydanio rerio* (embryos)	Henschel *et al.* (1997)
Paracetamol/ Acetaminophen	Analgesic; Anti-pyretic	134	72h EC_{50}	*Scenedesmus subspicatus*	Henschel *et al.* (1997)
Paroxetine HCl	Anti-depressant	3.0	EC_{50}	*Daphnia spp.*	FDA-CDER (1996)
Paroxetine HCl	Anti-depressant	2.0	LC_{50}	*Lepomis macrochirus*	FDA-CDER (1996)
Paroxetine HCl	Anti-depressant	3.29	4h LOEC	*Sphaerium* spp.	Fong *et al.* (1998)
Perindopril Erbumine	Anti-hypertensive	>1,000	EC_{50}	*Daphnia spp.*	FDA-CDER (1996)
Perindopril Erbumine	Anti-hypertensive	>990	LC_{50}	*Lepomis macrochirus*	FDA-CDER (1996)

Pentobarbital	Sedative; Hypnotic	49.5	96h EC_{50}	*Pimephales promelas*	Russom *et al.* (1997)
Phenobarbital	Anticonvulsant; Sedative; Hypnotic	484	96h EC_{50}	*Pimephales promelas*	Russom *et al.* (1997)
Phenobarbital (Phenobarbitone)	Anticonvulsant; Sedative; Hypnotic	>10,000	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Phenobarbital (Phenobarbitone)	Anticonvulsant; Sedative; Hypnotic	1,212	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Phenobarbital (Phenobarbitone)	Anticonvulsant; Sedative; Hypnotic	1,463	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Phenobarbital (Phenobarbitone)	Anticonvulsant; Sedative; Hypnotic	5,179	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Porfirmer Sodium	Photosensitiser	>994	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)

Propranolol HCl	Anti-hypertensive; Anti-anginal; Anti-arrhythmic	2.7	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
R-(±) Propranolol	Anti-hypertensive; Anti-anginal; Anti-arrhythmic	407	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
R-(±) Propranolol	Anti-hypertensive; Anti-anginal; Anti-arrhythmic	1.87	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
R-(±) Propranolol	Anti-hypertensive; Anti-anginal; Anti-arrhythmic	15.87	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
R-(±) Propranolol	Anti-hypertensive; Anti-anginal; Anti-arrhythmic	2.59	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)

Quinacrine HCl	Anthelminthic; Anti-malarial	122	48h LC_{50}	*Oncorhynchus mykiss*	Willford (1966)
Quinacrine HCl	Anthelminthic; Anti-malarial	25.0	24h LC_{50}	*Salvelinus namaycush*	Willford (1966)
Quinacrine HCl	Anthelminthic; Anti-malarial	21.0	48h LC_{50}	*Salvelinus namaycush*	Willford (1966)
Quinacrine HCl	Anthelminthic; Anti-malarial	300	24h LC_{50}	*Salmo trutta*	Willford (1966)
Quinacrine HCl	Anthelminthic; Anti-malarial	230	48h LC_{50}	*Salmo trutta*	Willford (1966)
Quinacrine HCl	Anthelminthic; Anti-malarial	196	24h LC_{50}	*Ictalurus punctatus*	Willford (1966)
Quinacrine HCl	Anthelminthic; Anti-malarial	70	48h LC_{50}	*Ictalurus punctatus*	Willford (1966)
Quinacrine HCl	Anthelminthic; Anti-malarial	230	48h LC_{50}	*Salvelinus fontinalis*	Willford (1966)
Quinacrine HCl	Anthelminthic; Anti-malarial	120	24h LC_{50}	*Lepomis macrochirus*	Willford (1966)
Quinacrine HCl	Anthelminthic; Anti-malarial	79	48h LC_{50}	*Lepomis macrochirus*	Willford (1966)
Quinacrine HCl	Anthelminthic; Anti-malarial	7.7	24h LC_{50}	*Penaeus setiferus*	Johnson (1976)
Quinidine Sulphate	Cardiac depressant (Anti-arrhythmic)	60	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)

Quinidine Sulphate	Cardiac depressant (Anti-arrhythmic)	274	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Quinidine Sulphate	Cardiac depressant (Anti-arrhythmic)	8.3	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Quinidine Sulphate	Cardiac depressant (Anti-arrhythmic)	60	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Quinidine Sulphate	Cardiac depressant (Anti-arrhythmic)	8.7	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Quinine Bisulphate	Anti-malarial; Oral sclerosing agent	13.1	24h LC_{50}	*Penaeus setiferus*	Johnson (1976)
Quinine HCl	Anti-malarial	>100	48h LC_{50}	*Oncorhynchus mykiss, Salmo trutta, Salvelinus fontinalis, Ictalurus punctatus, Lepomis macrochirus & Salvelinus namaycush*	Willford (1966)
Quinine Sulfate	Anti-malarial; Muscle relaxant	13.8	24h LC_{50}	*Penaeus setiferus*	Johnson (1976)

Ranitidine HCl	Anti-ulcerative	650	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Risperidone	Anti-psychotic	6.0	LC_{50}	*Lepomis macrochirus*	FDA-CDER (1996)
Risperidone	Anti-psychotic	6.0	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Salicylic Acid	Topical keratolytic	>1,440	24h EC_{50}	*D. magna*	Bringmann & Kühn (1982)
Salicylic Acid	Topical keratolytic	230	24h EC_{50}	*D. magna*	Wang & Lay (1989)
Salicylic Acid	Topical keratolytic	118	EC_{50}	*D. magna*	Henschel *et al.* (1997)
Salicylic Acid	Topical keratolytic	37.0	48h EC_{50}	*Brachydanio rerio* (embryos)	Henschel *et al.* (1997)
Salicylic Acid	Topical keratolytic	>100	72h EC_{50}	*Scenedesmus subspicatus*	Henschel *et al.* (1997)
Simethicone	Anti-flatulent	44.5	48h TL_{50}	*D.magna*	Hobbs (1975)
Salmeterol	Anti-asthmatic	20	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Secobarbital, sodium salt	Sedative, hypnotic	23.6	96h EC_{50}	*Pimephales promelas*	Russom *et al.* (1997)
Spirapril HCl	Anti-hypertensive	>930	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Spirapril HCl	Anti-hypertensive	>970	LC_{50}	*Lepomis macrochirus*	FDA-CDER (1996)
Stavudine	Anti-(retro)viral	>980	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)

Sulfadimethoxine	Anti-bacterial	1866	24h LC_{50}	*Artemia salina* (nauplii)	Brambilla *et al.* (1994)
Sulfadimethoxine	Anti-bacterial	851	48h LC_{50}	*Artemia salina* (nauplii)	Brambilla *et al.* (1994)
Sulfadimethoxine	Anti-bacterial	537	72h LC_{50}	*Artemia salina* (nauplii)	Brambilla *et al.* (1994)
Sulfadimethoxine	Anti-bacterial	19.5	96h LC_{50}	*Artemia salina* (nauplii)	Brambilla *et al.* (1994)
Sulfadimethoxine	Anti-bacterial	1,866	24h LC_{50}	*Artemia salina* (nauplii)	Migliore *et al.* (1993)
Sulfadimethoxine	Anti-bacterial	851	48h LC_{50}	*Artemia salina* (nauplii)	Migliore *et al.* (1993)
Sulfadimethoxine	Anti-bacterial	537	72h LC_{50}	*Artemia salina* (nauplii)	Migliore *et al.* (1993)
Sulfadimethoxine	Anti-bacterial	19.5	96h LC_{50}	*Artemia salina* (nauplii)	Migliore *et al.* (1993)
Sulfamerazine	Anti-bacterial	>100	48h LC_{50}	*Oncorhynchus mykiss, Salmo trutta, Salvelinus fontinalis, Ictalurus punctatus, Lepomis macrochirus & Salvelinus namaycush*	Willford (1966)

Sulfamethazine	Anti-bacterial	>100	48h LC_{50}	*Oncorhynchus mykiss, Salmo trutta, Salvelinus fontinalis, Ictalurus punctatus, Lepomis macrochirus & Salvelinus namaycush*	Willford (1966)
Sulfisoxazole	Anti-bacterial	>100	48h LC_{50}	*Oncorhynchus mykiss, Salmo trutta, Salvelinus fontinalis, Ictalurus punctatus, Lepomis macrochirus & Salvelinus namaycush*	Willford (1966)
Sumatriptan Succinate	Anti-migraine	290	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Tetracycline	Anti-amebic, Anti-bacterial, Anti-ricketettsial	16	72h EC_{50}	*Nitzschia closterium*	Peterson *et al.* (1993)
Tetracycline HCl	Anti-amebic, Anti-bacterial, Anti-ricketettsial	220	24/96h LC_{50}	*Salvelinus namaycush*	Marking *et al.* (1988)

Tetracycline HCl	Anti-amebic, Anti-bacterial, Anti-ricketettsial	>182	24/48/96 h LC_{50}	*Morone saxatilis*	Welborn (1969)
Theophylline	Bronchodilator	155	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Theophylline	Bronchodilator	8,247	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Theophylline	Bronchodilator	425	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Theophylline	Bronchodilator	483	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Theophylline	Bronchodilator	3,926	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Thiopental, sodium salt	Anesthetic	26.2	96h EC_{50}	*Pimephales promelas*	Russom *et al.* (1997)
Thiotepa	Anti-neoplastic	546	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)
Thioridazine HCl	Anti-psychotic	0.69	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Thioridazine HCl	Anti-psychotic	14.5	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Thioridazine HCl	Anti-psychotic	0.33	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Thioridazine HCl	Anti-psychotic	4.56	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Thioridazine HCl	Anti-psychotic	0.30	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)

Tiludronate Disodium	Metabolic Bone Disease	562	24h EC_{50}	*D. magna*	Sanofi (1996)
Tiludronate Disodium	Metabolic Bone Disease	320	48h EC_{50}	*D. magna*	Sanofi (1996)
Tolazoline HCl	Anti-adrenergic	354	96h EC_{50}	*Pimephales promelas*	Russom *et al.* (1997)
Tramadol HCl	Analgesic	130	LC_{50}	Unspecified fish	FDA-CDER (1996)
Tramadol HCl	Analgesic	73	EC_{50}	*Daphnia* spp	FDA-CDER (1996)
Verapamil HCl	Anti-anginal; Anti-arrhythmic	327	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Verapamil HCl	Anti-anginal; Anti-arrhythmic	356	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Verapamil HCl	Anti-anginal; Anti-arrhythmic	6.24	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Verapamil HCl	Anti-anginal; Anti-arrhythmic	55.5	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Verapamil HCl	Anti-anginal; Anti-arrhythmic	10.90	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Warfarin	Anti-coagulant	12	96h LC_{50}	*Rasbora heteromorpha*	Tooby *et al.* (1975)

Warfarin	Anti-coagulant	89	24h EC_{50}	*D. magna*	Lilius *et al.* (1994)
Warfarin	Anti-coagulant	3,638	24h LC_{50}	*Artemia salina*	Calleja *et al.* (1994a)
Warfarin	Anti-coagulant	342	24h LC_{50}	*Streptocephalus proboscideus*	Calleja *et al.* (1994a)
Warfarin	Anti-coagulant	475	24h EC_{50}	*D. magna*	Calleja *et al.* (1994a)
Warfarin	Anti-coagulant	444	24h LC_{50}	*Brachionus calyciflorus*	Calleja *et al.* (1994a)
Zalcitabine	Anti-(retro)viral	>1,790	EC_{50}	*Daphnia* spp.	FDA-CDER (1996)

The distribution of the acute data is presented in Table 2. In collating and summarising the data, the most sensitive species/endpoint and most toxic salt were chosen for any given drug active.

Table 2. - Summary of available acute ecotoxicity data for human pharmaceuticals.

Ecotoxicity Range	Number	Frequency (%)	Cumulative (%)
<0.1 mg/l	2	1.9	1.9
>0.1 - 1 mg/l	8	7.5	9.3
>1 - 10 mg/l	22	20.3	29.9
>10 - 100 mg/l	31	29.0	58.9
>100 - 1,000 mg/l	37	34.6	93.5
> 1,000 mg/l	7	6.5	100
Total	107	-	-

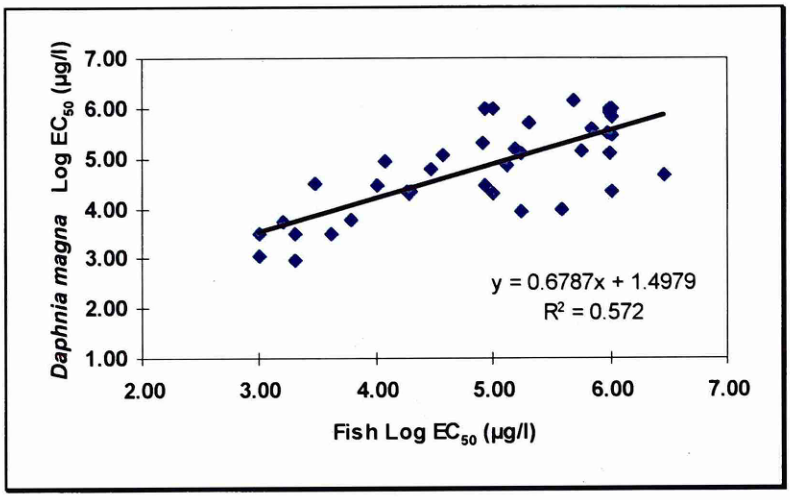
Comparisons of taxa sensitivities (in terms of acute responses) are presented in Table 3 and Figures 1 - 3. Fish, *Daphnia magna* and algae were chosen as representative of different trophic levels. Significant ($p<0.05$) correlations were observed between all taxa pairs with r values in the range 0.53 - 0.76. A sensitivity order algae > *Daphnia magna* > fish is consistent with the results of the regressions.

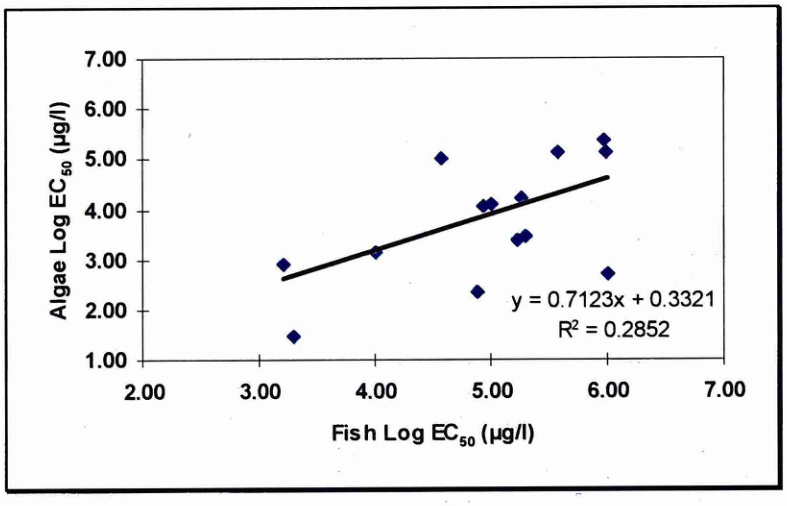
Table 3. - Paired comparison of relative taxa sensitivity.

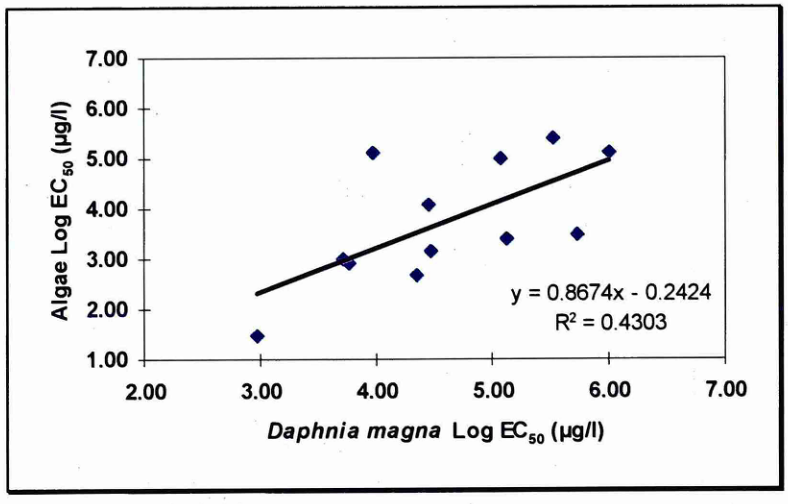
Comparison	Regression	r
Fish v *Daphnia magna* (n = 40)	Fish Log EC_{50} (µg/l) = *Daphnia magna* EC_{50} (µg/l) * 0.68 + 1.50	0.76
Fish v Algae (n = 15)	Fish Log EC_{50} (µg/l) = Algae EC_{50} (µg/l) * 0.71 + 0.33	0.53
Daphnia magna v Algae (n = 12)	*Daphnia magna* Log EC_{50} (µg/l) = Algae EC_{50} (µg/l) * 0.87 -0.24	0.66

$p<0.05$ in all cases. NB: Some algal endpoints are MIC rather than EC_{50}.

Figures 1 - 3. - Paired comparison of relative taxa sensitivity.

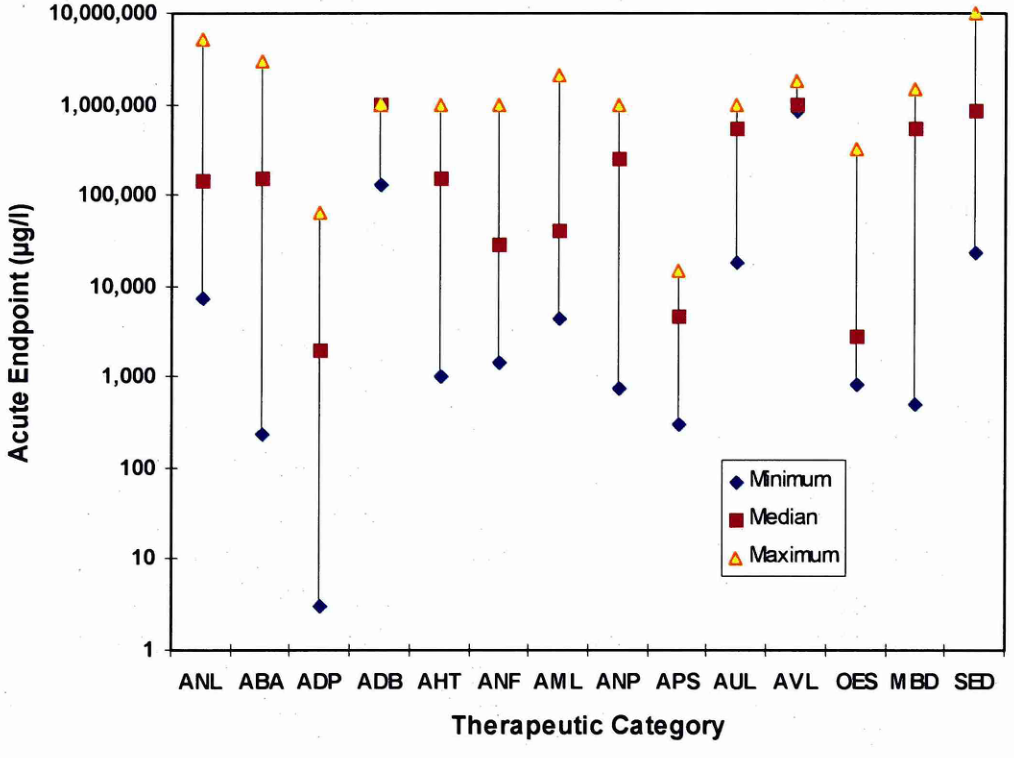






The median and range of acute endpoints for selected therapeutic categories for which there are two or more different compounds are presented in Figure 4. This allows some interpretation of the relative ecotoxicity of different classes of pharmaceuticals. The most ecotoxic of the various therapeutic classes of pharmaceuticals (in terms of observed minima) were anti-depressants, anti-bacterials and anti-psychotics, although the range of reported responses within each of these categories (and indeed most the other categories) was large i.e., typically over several orders of magnitude.

Figure 4. Median and range of acute endpoints for selected therapeutic categories.



Key: ANL = Analgesic, Anti-Inflammatory, Anti-Pyretic (7); ABA = Anti-Bacterial (18); ADP = Anti-Depressant (5 excluding Lithium); ADB = Anti-Diabetic (2); AHT = Anti-Hypertensive, Anti-Anginal, Anti-Arrhythmic (9); ANF = Anti-Infective (4); AML = Anti-Malarial (4); ANP = Anti-Neoplastic (4); APS = Anti-Psychotic (2); AUL = Anti-Ulcerative (5); AVL = Anti-Viral (4); OES = Oestrogen (2); MBD = Metabolic Bone Disease (3); SED = Sedative/Hypnotic (4). Figures in brackets denote the number of compounds.

Chronic Ecotoxicity Data

The available chronic ecotoxicity database is presented in Table 4. Most endpoints were determined via standard tests with *D. magna* and algae. Other more sensitive endpoints for Ethinyl Oestradiol relate to the induction of plasma vitellogenin, gonadosomatic index (GSI) and spermatogenesis in roach (*Rutilus rutilus*) and/or rainbow trout (*Oncorhynchus mykiss*) (FWR, 1992; Purdom *et al.*, 1994; FWR, 1995; Jobling *et al.*, 1996).

Table 4. - Chronic ecotoxicity data for human pharmaceuticals.

Compound	Category	Value (mg/l)	Endpoint/ Duration	Species	Reference
Alendronate Sodium	Metabolic Bone Disease	0.5	NOEC	Unspecified green algae	FDA-CDER (1996)
Bicalutamide	Non-steroidal Anti-androgen	1	NOEC	Unspecified blue-green algae	FDA-CDER (1996)
Bicalutamide	Non-steroidal Anti-androgen	1	NOEC	Unspecified green algae	FDA-CDER (1996)
Budenoside	Anti-inflammatory	10	NOEC	Unspecified green algae	FDA-CDER (1996)
Cisapride	Peristaltic Stimulant	100	"*Effects*"	Unspecified blue-green algae	FDA-CDER (1996)
Cisapride	Peristaltic Stimulant	320	"*Effects*"	Unspecified green algae	FDA-CDER (1996)

Clofibrate	Antihyper-lipoproteinemic	0.01 [A/C 1428)	21d Repro-duction NOEC	*D. magna*	Köpf (1995)
Clofibrate	Antihyper-lipoproteinemic	0.0084	21d Repro-duction EC_{10}	*D. magna*	Köpf (1995)
Clofibrate	Antihyper-lipoproteinemic	0.106	21d Repro-duc-tion EC_{50}	*D. magna*	Köpf (1995)
Clofibrate	Antihyper-lipoproteinemic	5.4	EC_{10}	Unspecified algae	Köpf (1995)
Diethylstilbestrol	Estrogen	0.25/0.5	F_1 21d molt frequency NOEC/ LOEC	*D. magna*	Baldwin *et al.* (1995)
Diethylstilbestrol	Estrogen	0.062/0.5 (A/C 17.6)	F_2 21d brood size NOEC/ LOEC	*D. magna*	Baldwin *et al.* (1995)
Diethylstilbestrol	Estrogen	0.01	21d repro-duction NOEC	*Tisbe batta-gliai*	Hutchinson *et al.* (1999)

Ethinyl Oestradiol	Estrogen	1 ng/l	10d Plasma vitellogenin NOEC (9.5°C)	*Rutilus rutilus*	FWR (1992)
Ethinyl Oestradiol	Estrogen	1 ng/l	10d Plasma vitellogenin NOEC (9.5°C)	*Oncorhynchus mykiss*	FWR (1992)
Ethinyl Oestradiol	Estrogen	0.3 ng/l	28wk Plasma vitellogenin LOEC	*Oncorhynchus mykiss*	Sheahan *et al.* (1994)
Ethinyl Oestradiol	Estrogen	0.1 ng/l	10d Plasma vitellogenin LOEC (16.5°C)	*Oncorhynchus mykiss*	Purdom *et al.* (1994)
Ethinyl Oestradiol	Estrogen	0.01 (A/C 570)	21d Reproduction NOEC	*D. magna*	Köpf (1995)
Ethinyl Oestradiol	Estrogen	0.0125	21d Reproduction EC_{10}	*D. magna*	Köpf (1995)
Ethinyl Oestradiol	Estrogen	0.105	21d Reproduction EC_{50}	*D. magna*	Köpf (1995)

Ethinyl Oestradiol	Estrogen	0.054	EC_{10}	Unspecified algae	Köpf (1995)
Ethinyl Oestradiol	Estrogen	2 ng/l (A/C 800,000)	21d Spermatogenesis, GSI & plasma vitellogenin LOEC	*Oncorhynchus mykiss*	FWR (1995)
Ethinyl Oestradiol	Estrogen	1 ng/l	21d Plasma vitellogenin (positive control for AP)	*Rutilus rutilus*	FWR (1995)
Ethinyl Oestradiol	Estrogen	2 ng/l	21d Spermatogenesis, GSI & plasma vitellogenin (positive control for AP)	*Oncorhynchus mykiss*	Jobling *et al.* (1996)
Ethinyl Oestradiol	Estrogen	0.387	21d Reproduction LOEC	*D. magna*	Schweinfurth *et al.* (1996)

Ethinyl Oestradiol	Estrogen	10 ng/l	28d Reproduction LOEC	*Pimephales promelas*	Schweinfurth *et al.* (1996)
Ethinyl Oestradiol	Estrogen	1.25 ng/l	50-60d LOEC (growth)	*Lymnaea stagnalis*	Belfroid & Leonards (1996)
Ethinyl Oestradiol	Estrogen	0.125 ng/l	50 - 60d LOEC (growth)	*Bithynia tentaculata*	Belfroid & Leonards (1996)
Ethinyl Oestradiol	Estrogen	1 ng/l	9 month Reproduction NOEC (growth retardation LOEC 4 ng/l)	*Pimephales promelas*	Länge *et al.* (1997); Länge *et al.* (2001).
Etidronic Acid	Metabolic Bone Disease	>12 (A/C 43.9)	28d NOEC	*D. magna*	Gledhill & Feijtel (1992)
Etidronic Acid	Metabolic Bone Disease	1.3	96h NOEC	*Selenastrum* spp.	Gledhill & Feijtel (1992)
Etidronic Acid	Metabolic Bone Disease	13.2	14d NOEC	*Selenastrum* spp.	Gledhill & Feijtel (1992)

Finasteride	Treatment of benign prostatic hy-pertrophy	≥49	NOEC	Unspecified green algae	FDA-CDER (1996)
Fluoxetine HCl	Anti-depressant	0.001	NOEC	Unspecified green algae	FDA-CDER (1996)
Fluvoxamine Male-ate	Anti-depressant	31	NOEC	Unspecified green algae	FDA-CDER (1996)
Iopromide	Diagnostic Aid (radiopaque medium)	>1,000 (A/C 1.0)	21d Repro-duction NOEC	*D. magna*	Schweinfurth *et al.* (1996a)
Iopromide	Diagnostic Aid (radiopaque medium)	68	NOEC	Unspecified blue-green al-gae	FDA-CDER (1996)
Lomefloxacin	Anti-bacterial	2	NOEC	Unspecified green algae	FDA-CDER (1996)
Lorcarbef	Anti-infective	13	NOEC	Unspecified green algae	FDA-CDER (1996)
Losartan K	Anti-hypertensive	556	NOEC	Unspecified blue-green al-gae	FDA-CDER (1996)
Losartan K	Anti-hypertensive	143	NOEC	Unspecified green algae	FDA-CDER (1996)
Metronidazole	Anti-protozoal	19.9	72h EC_{10}	*Selenastrum capricornutum*	Lanzky & Halling-Sørenson (1997)

Metronidazole	Anti-protozoal	2.03	72h EC_{10}	*Chlorella* sp.	Lanzky & Halling-Sørenson (1997)
Nicotine	Cholinergic agonist	0.07 (A/C 42.9)	LOEC (length)	*D. pulex*	FDA-CDER (1996)
Riseperidone	Anti-psychotic	100	"*Effects*"	Unspecified blue-green algae	FDA-CDER (1996)
Riseperidone	Anti-psychotic	10	"*Effects*"	Unspecified green algae	FDA-CDER (1996)
Salicylic Acid	Topical keratolytic	<20.0 (A/C 5.9)	21 d Reproduction NOEC	*D. magna*	Wang & Lay (1989)
Tiludronate Disodium	Metabolic Bone Disease	36.6	14d EC_{50}	*Selenastrum capricornutum*	Sanofi (1996)
Tiludronate Disodium	Metabolic Bone Disease	13.3	21d EC_{50}	*Microcystis aeruginosa*	Sanofi (1996)

DISCUSSION

Acute ecotoxicity data are available for a large number of pharmaceuticals (i.e., >100). The results presented here possibly represents the most comprehensive dataset yet collated. Most of the data relate to acute ecotoxicity endpoints, although some chronic data are available. The range of reported acute ecotoxicity endpoints varied from >15,000 mg/l for Atropine Sulphate (Anti-cholinergic/ mydriatic) in a standard 24 hour LC_{50} *Artemia salina* test (Calleja *et al.* 1994a) down to 0.003 mg/l for Fluvoxamine (an antidepressant) in a (non-

standard) study examining the effects of selective serotonin re-uptake inhibitors (SSRIs) upon parturition (release of juveniles) in fingernail clams (Fong *et al.*, 1998). This corresponds to a difference of 6 orders of magnitude. Ten of the compounds had acute endpoints of ≤1 mg/l. They were Alendronate (a biphosphonate used in the treatment of metabolic bone disease), Amitriptyline (an anti-depressant), Carvedilol (an anti-hypertensive and anti-anginal), Ethinyl Oestradiol (an oestrogen), Fluticasone (a corticosteroid anti-asthmatic), Fluoxetine (an anti-depressant), Fluvoxamine (an anti-depressant), Midazolam (an anesthetic), Paclitexel (an anti-neoplastic) and Thioridazine (an anti-psychotic).

In a similar review exercise, the US FDA Center for Drug Evaluation and Research (CDER) has performed a retrospective review of toxicity information available in Environmental Assessments (EA) previously submitted in support of New Drug Applications (NDA) (FDA-CDER, 1996). The data showed no observed effects on relevant standard environmental test organisms at drug concentrations below 1 µg/l (based on both acute and chronic data from approximately 60 compounds). The results of the FDA-CDER review provided the justification for the 1 µg/l cut-off threshold employed in the US FDA environmental risk assessment framework for pharmaceuticals (Federal Register 29/07/97 Volume 62, p40569). All acute ecotoxicity endpoints considered in this study (which includes the data from the FDA-CDER review) were similarly also >1 µg/l. That the majority of the pharmaceuticals examined are limited (90^{th}-percentile >1mg/l) in their acute ecotoxicity is not surprising, given the generally limited mammalian toxicity required of pharmaceuticals. A relationship between mammalian toxicity and invertebrate ecotoxicity of industrial chemicals and pharmaceuticals has similarly been noted elsewhere (e.g., Enslein *et al.*, 1987; Enslein *et al.*, 1989; Calleja *et al.*, 1993; Calleja *et al.*, 1994b). For perspective, the EU classification criteria for risk phrases (67/548/EEC) defines compounds with an $L(E)C_{50}$ ≤1 mg/l as "*very toxic to aquatic organ-*

isms" (R-50), 1 - 10 mg/l as "*toxic to aquatic organisms*" (R-51) and 10 - 100 mg/l as "*harmful to aquatic organisms*" (R-52).

The comparisons of paired taxa in terms of responses to acute ecotoxicity testing suggests a general hierarchy of sensitivity corresponding to algae > *Daphnia magna* > fish. Nevertheless, where differences in responses are observed they are typically limited to one order of magnitude. The average difference between fish and *Daphnia magna* is <0.5 log units, between fish and algae 1.2 log units and between *Daphnia magna* and algae 1.0 log units. Relative algal sensitivity may have resulted in part from the effects of anti-infectives and anti-bacterials (such as Oxytetracycline) upon algae.

The most ecotoxic of the various therapeutic classes of pharmaceuticals (in terms of observed minima) were anti-depressants, anti-bacterials and anti-psychotics. The lowest endpoint (3 µg/l) for anti-depressants relates to a test in a freshwater bivalve (Fong *et al.*, 1998). Even without the inclusion of this non-standard endpoint, anti-depressants would remain the most potent therapeutic class in terms of acute ecotoxicity by virtue of an algal EC_{50} of 31 µg/l for Fluoxetine (FDA-CDER, 1996)[10]. As indicated above, the effects of anti-bacterials upon algae and in particular blue-green algae (cyanobacteria) accounts for the extension of the (lower) range of reported responses for anti-bacterials. After anti-bacterials, the next lowest minima of any category is that of anti-psychotics. In addition, it is also worth noting that the potency of oestrogens in the acute tests reviewed here is reflected in the relatively low median value for this category. Variation within each of these categories (and indeed most the other categories) was large i.e., typically over several orders of magni-

[10] There are structural similarities between Fluoxetine and certain phenoxy herbicides such as Fluazifo-p-butyl, Mecoprop or Difenopenten that may help to explain the apparent sensitivity of microalgae.

tude. This presumably reflects the variation in responses of the differing taxa/trophic levels (i.e., fish, invertebrates or algae) when exposed to representative compounds from the various differing therapeutic classes.

The overall applicability of acute ecotoxicity data in general for environmental risk assessment purposes has been criticised (Hallling-Sørenson *et al.*, 1998). Standard acute bioassays with their focus on immediate endpoints such as lethality may not be the most appropriate basis for risk assessment given the intended narrow scope of biological activity/effect and general potency of pharmaceuticals in general. It has consequently been suggested that chronic bioassays performed over the life-cycle of various organisms from different trophic levels may be more appropriate (Halling-Sørenson *et al.*, 1998).

Within this study, chronic ecotoxicity data for aquatic organisms were secured for 20 compounds. The chronic database itself is dominated by data relating to Ethinyl Oestradiol. The various endpoints reported for Ethinyl Oestradiol demonstrates the exquisite potency of this compound. The ecological significance of some of the various biomarker responses reported for Ethinyl Oestradiol is not known and they are less readily employed in risk characterisation. This contrasts with the more integrative fathead minnow reproduction LOEC/NOEC endpoints for Ethinyl Oestradiol reported by Schweinfurth *et al.* (1996) and Länge *et al.* (1997; 2001). The issue of ecological significance of biomarker responses for Ethinyl Oestradiol is discussed further in Länge *et al.* (2001).

The chronic database for the remaining compounds is dominated by algal endpoints (i.e., NOEC or EC_{10} values). Chronic toxicity studies relating to fauna (namely *Daphnia magna* or *pulex*) are limited to Clofibrate, Diethylstilbestrol, Etidronic Acid, Iopromide, Nicotine and Salicylic Acid. Acute EC_{50}/Chronic NOEC (A/C) ratios have been calculated for *Daphnia* spp.

(Table 4). Acute/Chronic ratios varied from 1 for Iopromide to 1,428 for Clofibrate with a median of 43 (n = 7). This does not contrast markedly with A/C ratios in the range of 1.6 to 1,030 (median 22.1) previously reported for invertebrates for industrial chemicals (ECETOC, 1993). Whilst not normally considered, A/C ratios can also be calculated for algae (EC_{50}/NOEC). In this study values were typically approximately 2 and were limited in all cases to one order of magnitude.

Only one example of an Acute/Chronic ratio was available for a fish species (*Oncorhynchus mykiss*) and pertains to Ethinyl Oestradiol[11]. The A/C ratio was 800,000 and reflects the marked difference in the magnitude of the observed endpoints in the respective acute and chronic bioassays that results from the endocrine modality of this compound. Such an observation could be employed as the basis of an argument that would preclude the use of short-term ecotoxicity testing for the purposes of risk assessment of endocrinologically active compounds and oblige chronic testing preferably with vertebrates (i.e., fish). The lack of comparative acute and chronic data relating to fish for other (non-oestrogenic) pharmaceuticals precludes the calculation of A/C ratios for such compounds.

CONCLUSIONS

Data relating to the effects of human pharmaceuticals upon aquatic organisms are available, although the majority relates to short-term acute responses such as lethality. A review revealed over 360 endpoints in macro-invertebrates, fish and algae for 107 compounds. Over 90% of the observations were at concentrations >1 mg/l suggesting the relative limited acute ecotoxicity of pharmaceuticals in general. All values were > 1 µg/l. It is interesting to note

[11] Additional data are also presented in OECD (2000). A/C ratios for Medaka (42d) and Zebra fish (300 d) for Ethinyl Oestradiol are 150,000 and 5,730,000 respectively.

that the majority of compounds with endpoints in acute bioassays of <1 mg/l are pharmaceuticals intended to impact the human nervous system (i.e., anti-depressant, anti-psychotic or anesthetic). The relative sensitivity of tested taxa to pharmaceuticals was algae > *Daphnia magna* > fish. Although this trend may reflect the impact of some compounds with intended biocidal modes of action (e.g., antibiotics) when tested upon algae. Differences in the responses of different taxa to the same compound were typically limited to one order of magnitude.

The applicability of the acute ecotoxicity database for environmental risk assessment purposes has been criticised on the basis of the appropriateness of the focus upon immediate endpoints such as lethality. Pharmaceuticals are intended to have a narrow scope of biological effect and it has been suggested that chronic testing may therefore be more appropriate. The available chronic ecotoxicity database is more limited and data for only 20 compounds are available. The chronic database was dominated by studies upon Ethinyl Oestradiol. The remaining endpoints were mostly concerned with algae or *Daphnia* spp. Acute/Chronic ratios for *Daphnia magna* and algae were calculated and do not differ markedly from those reported elsewhere for industrial chemicals. Whilst the scientific basis for the use of application factors in risk assessment to derive the PNEC (predicted No-Effect Concentration) from acute ecotoxicity data is not contraindicated by the A/C ratios observed for *Daphnia* or algae for pharmaceuticals, the absence of relevant chronic data precludes the derivation of A/C ratios for fish and a categorical conclusion vis-à-vis the applicability of current risk assessment practice to pharmaceuticals. More work relating to the potential chronic effects of pharmaceuticals in general and upon fish in particularly is required.

REFERENCES

Anon (1993) Acute toxicity to Bluegill (*Lepomis macrochirus*) of the test substance Ketorolac Tromethamine from Radian Corporation in a 96-hr static non-renewal test. Performed for Radian Corporation by AnaltiKEM Environmental Lab (Houston, USA). AnalytiKEM Test Number 01628.

Baldwin WS, Milam DL, Leblanc GA (1995) Physiological and biochemical perturbations in *Daphnia magna* following exposure to the model environmental estrogen diethylstilbestrol. *Environmental Toxicology & Chemistry* 14(6): 945-952.

Belfroid A, Leonards P (1996) Effect of Ethinyl oestradiol on the development of snails and amphibians. SETAC 17th Annual Meeting November 1996 Washington DC [Abstract PO/508].

Brambilla G, Civitareale C, Migliore L (1994) Experimental toxicity and analysis of bacitracin, flumequine and sulphadimethoxine in terrestrial and aquatic organisms as a predictive model for ecosystem damage. *Qumica Analitica* 13(Suppl. 1): S73-S77.

Bringmann G, Kühn R (1982) Ergebnisse der Schadwirkung wassergefährdender Stoffe gegen *Daphnia magna* in einem weiterentwickelten standardisierten Testverfahren [Results of the harmful effects of water pollutants to *Daphnia magna* in a further developed standardized test procedure]. *Z. Wasser Abwasser Forsch.* 15(1): 1-6.

Budavari, S (ed.) (1989) The Merck Index - An Encyclopedia of Chemicals, Drugs and Biologicals (11th Edition). Merck & Co. Inc. Rahway, N.J. (USA).

Calleja MC, Personne G, Geladi P (1993) The predictive potential of a battery of ecotoxicological tests for human acute toxicity, as evaluated with the first 50 MEIC chemicals. *ATLA* 21: 330-349.

Calleja MC, Personne G, Geladi P (1994a) Comparative acute toxicity of the first 50 Multicentre Evaluation of *In Vitro* Cytotoxicity chemicals to aquatic non-vertebrates. *Arch. Environ. Contam. Toxicol.* 26: 69-78.

Calleja MC, Geladi P, Personne G (1994b) Modelling of human acute toxicity from physicochemical properties and non-vertebrate acute toxicity of the 38 organic chemicals of the MEIC priority list by PLS regression and neural network. *Fd. Chem. Toxic.* 32(10): 923-941.

Coats JR, Metcalf RL, Lu P-Y, Brown DD, Williams, JF and Hansen LG (1976) Model ecosystem evaluation of the environmental impacts of the veterinary drugs phenothiazine, sulfametazine, clopidol and diethylstibestrol. *Envir. Health Perspect.* 1: 167-197.

Di Delupis GD, Macri A, Civitareale C, Migliore L (1992) Antibiotics of zootechnical use: effects of acute high and low dose contamination on *Daphnia magna* Straus. *Aquatic Toxicology* 22: 53-60.

ECETOC (1993). *Aquatic Toxicity Data Evaluation* [Technical Report No. 56]. European Centre for Ecotoxicology & Toxicity of Chemicals: Brussels.

Enslein K, Tuzzeo TM, Borgstedt HH, Blake BW, Hart JB (1987) Prediction of rat oral LD_{50} from *Daphnia magna* LC_{50} and chemical structure: In: Kaiser KLE (ed) *QSAR in Environmental Toxicology - II.* DD Reidel Publishing Company, Dordrecht, pp 91-106.

Enslein K, Tuzzeo TM, Blake BW, Hart JB, Landis WG (1989) Prediction of *Daphnia magna* EC_{50} values from rat oral LD_{50} and structural parameters: In: Suter GW, Lewis MA (eds) *Aquatic Toxicology and Environmental Fate: 11th Volume ASTM STP 1007.* American Society for Testing & Materials, Philadelphia (PA, USA), pp 397-409.

FDA-CDER (1996). *Retrospective Review of Ecotoxicity Data Submitted in Environmental Assessessments [Docket No. 96N-0057].* FDA Center for Drug Evaluation and Research: Rockville (MD), USA.

Fong PP, Huminski PT, D'Urso LM (1998) Induction and potentiation of parturition in Fingernail Clams (*Sphaerium striatinum*) by selective serotonin re-uptake inhibitors (SSRIs). *The Journal of Experimental Zoology* 280: 260-264.

FWR (1992) *Effects of Trace Organics on Fish [October 1992 FR/D 0008].* Foundation for Water Research, Marlow (Bucks.), UK

FWR (1995) *Effects of Trace Organics on Fish - Phase 2 [July 1995 FR/D 0022].* Foundation for Water Research, Marlow (Bucks.), UK

Gledhill WE, Feijtel, TCJ (1992) Environmental properties and safety assessment of organic phosphonates used for detergent and water treatment: In: de Oude NT (ed) NT *Detergents - Handbook of Environmental Chemistry Volume 3 Part F, Anthropogenic Compounds.* Springer-Verlag, New York, Berlin & Heidelberg, pp 261-285.

Halling-Sørenson B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten-Lützhøft HC, Jørgensen SE (1998) Occurrence, fate and effects of pharmaceutical substances in the environment - A review. *Chemosphere* 36(2): 357-393.

Henschel KP, Wenzel A, Diederich M, Fliedner, A (1997) Environmental Hazard Assessment of Pharmaceuticals. *Regulatory Toxicology & Pharmacology* 25: 220-225.

Hobbs EJ (1975) Toxicity of polydimethylsiloxanes in certain environmental systems. *Environmental Research* 10: 397-406.

Holten-Lützhøft H-C, Halling-Sorensen B, Jorgensen, S.E. (1998). Algal testing of antiobiotics applied in Danish fish farming. SETAC-Europe 8th Annual Meeting 14-18th April 1998 Bordeaux [Abstract 4I/004].

Hughes JS (1973) *Acute Toxicity of Thirty Chemicals to Stripped Bass (Morone saxatilis).* Presented at the Western Association of State Game and Fish Commissioners in Salt Lake City, Utah July 1973.

Hutchinson TH, Pounds NA, Hampel M, Williams TD (1999) Life-cycle studies with marine copepods (*Tisbe battagliai*) exposed to 20-hydroxyecdysone and diethylstilbestrol. *Environmental Toxicology & Chemistry* 18(12): 2914-2920.

Jobling S, Sheahan D, Osborne J., Matthiessen P, Sumpter JP (1996) Inhibition of testicular growth in rainbow trout (Oncorhynchus mykiss) exposed to estrogenic alkylphenolic chemicals. *Environmental Toxicology & Chemistry* 15(2): 194-202.

Johnson SK (1976) *Twenty-Four Hour Toxicity Tests of Six Chemicals to Mysis Larvae of Penaeus setiferus.* Texas A & M University Extension Disease Laboratory, Publication No. FDDL-S8.

Knoll/BASF (1995) Pharmaceutical safety data sheet (Issue/Revision 06/04/94). Knoll Pharmaceuticals, Nottingham, UK. Quoted in Halling-Sørenson *et al.* (1998).

Köpf W (1995) Wirkung endokriner Stoffe in Biotests mit Wasserorganismen. Vortag bei der 50. Fachtagung des Bayerisches Landesamt für Wasserwirtschaft: Stoffe mit endokriner Wirkung im Wasser (Abstract). [Effects of endocrine substances in bioassays with aquatic organisms. Presentation at the 50th Seminar of the Bavarian Association for Waters Supply. Substances with endocrine effects in water (Abstract)]. Quoted in Römbke *et al.* (1995).

Kühn R, Pattard, M, Pernak KD, Winter A (1989) Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to *Daphnia magna. Water Research* 23(4): 495-499.

Länge R, Hutchinson TH, Croudace CP, Siegmund F, Schweinfurth H, Hampe P, Panther GH, Sumpter JP (2001) Effects of the synthetic oestrogen 17α-ethinylestradiol over the life-cycle of the fathead minnow (*Pimephales promelas*). *Environmental Toxicology & Chemistry* 20(6): 1216-1227.

Länge R, Schweinfurth H, Croudace C, Panther G (1997) Growth and reproduction of fathead minnow (*Pimephales promelas*) exposed to the synthetic steroid hormone Ethinylestradiol in a life cycle test (Abstract). Seventh Annual Meeting of SETAC- Europe, Amsterdam, The Netherlands, April 6 - 10, 1997.

Lanzky PF, Halling-Sørenson B, (1997) The toxic effect of the antibiotic metronidazole on aquatic organisms. *Chemosphere* 35(11): 2553-2561.

Lilius H, Isomaa B, Holmström T (1994) A comparison of the toxicity of 50 reference chemicals to freshly isolated rainbow trout hepatocytes and *Daphnia magna*. *Aquatic Toxicology* 30: 47-60.

Macrì A, Sbardella E (1984) Toxicological evaluation of Nitrofurazone and Furazolidone on *Selenastrum capricornutum*, *Daphnia magna* and *Musca domestica*. *Ecotoxicol. Envir. Safety* 8: 115-105.

Marking L., Howe GE, Crowther JR (1988) Toxicity of erythromycin, oxytetracycline and tetracycline administered to Lake Trout in water baths, by injection or by feeding. *The Progressive Fish-Culturist* 50: 197-201.

Migliore L, Brambilla G, Grassitellis A, Di Delupis GD (1993) Toxicity and bioaccumulation of sulphadimethoxine in *Artemia* (*Crustacea, Anostraca*). *Int. J. Salt Lake Res.* 2(2): 141-152.

Migliore L, Civitareale C, Brambilla G, Di Delupis GD (1997) Toxicity of several important agricultural antibiotics to *Artemia*. *Wat. Res.* 31(7): 1801-1806.

OECD (2000) Record from the 2nd OECD Expert Consultation on Endocrine Disruptors Testing in Fish (EDF2) Tokyo 15-16th March 2000. Test Guidelines Programme Endocrine Disruptors Testing and Assessment Task Force (June 2000).

Panter GH, Thompson RS, Beresford N, Sumpter JP (1999) Transformation of a non-oestrogenic steroid metabolite to an oestrogenically active substance by minimal bacterial activity. *Chemosphere* 38(15): 3579-3596.

Peterson SM, Batley GE, Scammell, MS (1993) Tetracycline in antifouling paints. *Marine Pollution Bulletin* 26(2): 96-100.

Purdom CE, Hardiman PA, Bye VJ, Eno NC, Tyler CR, Sumpter JP (1994) Estrogenic effects of effluents from sewage treatment works. *Chemistry & Ecology* 8: 275-285.

Rodriguez C, Chellman K, Gomez S, Marple L (1992) Environmental assessment report persuant to 21 CFR 25.31(a) submitted to the US FDA in support of the New Drug Application (NDA) for naproxen for over-the-counter use. Hamilton Pharmaceuticals Limited: Puerto Rico.

Römbke J et al. (1996) Minutes of the Round Table Discussion: Medicines in the Environment held at the Federal German Bureau of the Environment (Berlin) on 15th December 1995 on behalf of the Federal German Bureau of the Environment (UBA).

Russom CL, Bradbury SP, Broderius SJ, Hammermeister DE, Drummond RA (1997) Predicting modes of toxic action from chemical structure: Acute toxicity in the fathead minnow (Pimephales promelas). *Environmental Toxicology & Chemistry* 16(5): 948-967.

Sanofi (1996). Tiludronate Disodium Material Safety Data Sheet SR 41319B Sanofi Research.

Schweinfurth H, Länge R, Günzel P (1996) Environmental fate and ecological effects of steroidal estrogens. Presentation at the "*Oestrogenic Chemicals in the Environment*" conference organised by IBC Technical Services Ltd in London on the 9th & 10th May, 1996.

Schweinfurth H, Länge R & Schneider PW (1996a) Environmental risk assessment in the pharmaceutical industry. Presentation at the "*3rd Eurolab Symposium - Testing and Analysis for Industrial Competitiveness and Sustainability*" Berlin, 5 - 7th June, 1996.

Sheahan DA, Bucke D, Matthiessen P, Sumpter JP, Kirby MF, Neall P, Waldock M (1994) The effects of low levels of 17 α-ethynylestradiol upon plasma vitellogenin levels in male and female rainbow trout, Oncorhynchus mykiss, held at two acclimation temperatures. In: Müller R, Lloyd R (eds) *Sublethal and Chronic Effects of Pollutants on Freshwater Fish.* Fishing News Books, Blackwell Science, Oxford, 99-112.

Tooby TE, Hursey PA, Alabaster JS (1975) The acute toxicity of 102 pesticides and miscellaneous substances to fish. *Chemistry and Industry* 21st June 1975: 523-526.

Wang WH, Lay JP (1989) Fate and effects of salicylic acid compounds in freshwater systems. *Ecotoxicology and Environmental Safety* 17(3): 308-316.

Welborn TL (1969) The toxicity of nine therapeutic and herbicidal compounds to stripped bass. *The Progressive Fish Culturist* 31(1): 27-32.

Wilford WA (1966) *Toxicity of 22 Therapeutic Compounds to Six Fishes.* US Dept. of the Interior, Fish & Wildlife Service, Bureau of Sports Fisheries & Wildlife - Resource Publication 35. Washington DC December 1966.

Zou E, Fingerman (1997) Synthetic estrogenic agents do not interfere with sex differentiation but do inhibit molting of the cladoceran *Daphnia magna. Bull. Environ. Contam. Toxicol.* 58: 596-602.

APPENDIX

Fish:

Brachyderio (=Danio) rerio - Zebra fish

Gambusia affinis - Mosquito fish

Ictalurus punctatus - Channel catfish

Lepomis macrochirus - Bluegill sunfish

Morone saxatilis - Striped bass

Oncorhynchus mykiss - Rainbow trout

Pimephales promelas - Fathead minnow

Rasbora heteromorpha - Harlequin fish

Rutilus rutilus - Roach

Salmo trutta - Brown trout

Salvelinus fontinalis - Brook trout

Salvelinus namaycush - Lake trout

Invertebrates:

Acartia tonsa - Copepod crustacean

Artemia salina - Anostracan crustacean

Brachionus calyciflorus - Rotifer

Bithynia tentaculata - Gastropod mollusc

Daphnia magna - Cladoceran crustacean (Water flea)

Hyalella azteca - Amphipod crustacean

Lymnaea stagnalis - Gastropod mollusc (Pond snail)

Panaeus setiferus - Decapod crustacean (White shrimp)

Physa sp. - Gastropod mollusc (Bladder Snail)

Sphaerium striatinum - Bivalve mollusc (Fingernail clam)

Streptocephalus proboscideus - Anostracan crustacean

Tisbe battagliai – Copepod crustacean

Algae:

Scenedesmus subspicatus - Green algae

Selenastrum capricornutum - Green algae

Nitzschia closterium - Marine diatom

Skeletonema costatum - Marine diatom

Chlorella spp. - Green algae

Microcystis aeruginosa - Blue-green algae

CHAPTER 4

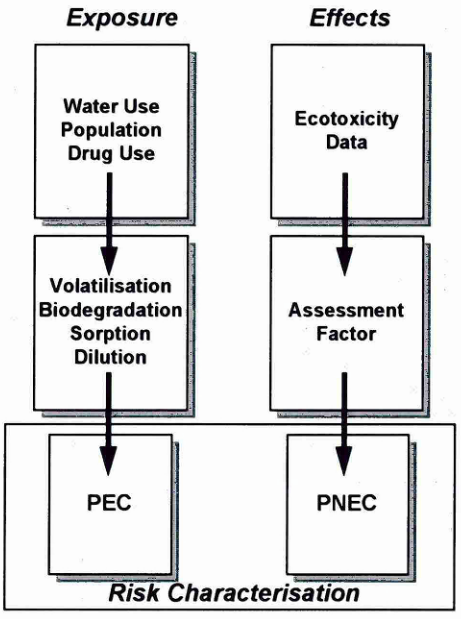
AQUATIC RISK CHARACTERISATION

This chapter has been published in "*Pharmaceuticals in the Environment – Sources, Fate, Effects and Risks*" (Ed. K. Kümmerer) under the title "*A Data Based Perspective on the Environmental Risk Assessment of Human Pharmaceuticals II – Aquatic Risk Assessment*" pp 203- 219 (Springer, 2001).

INTRODUCTION

Environmental risk assessment (ERA) evaluates the likelihood that adverse ecological effects result from exposure to a substance. It therefore requires a consideration of both exposure and effects in relevant environmental compartments. The exposure assessment considers the fate of a substance released to the environment and predicts the environmental concentration or PEC ("*Predicted Environmental Concentration*"). The effects assessment considers data relating to the effects of the substance upon representative biota and uses such data to predict the no-effect concentration or PNEC ("*Predicted No-Effect Concentration*") for the various environmental compartments (i.e., surface waters, sediment, soil, etc.). The PEC and PNEC are combined in order to characterise the risk, i.e., calculation of the PEC/PNEC ratio (see Figure 1). Decisions regarding the safety of the substance depend upon the value of this quotient.

Figure 1. - Risk assessment framework within the aquatic environment for human pharmaceuticals.



Risk assessment conventionally proceeds in an iterative/tiered process, employing simple and conservative assumptions to estimate PEC and PNEC at initial tiers and progressing through subsequent tiers by employing more realistic or representative assumptions when estimating

PEC and PNEC. Conservatism is incorporated into both the PEC (via the assumptions used to estimate the exposure) and PNEC (via use of assessment factors to extrapolate from laboratory derived-data to the ecosystem). The exposure and effects assessments do not have to simultaneously progress to successive tiers, and effort can be focused on those data that potentially have the largest impact upon the risk quotient or will reduce uncertainties.

If the environmental concentration in a compartment is less than the concentration causing "*no-effect*" to that compartment, i.e., PEC/PNEC <1, then it is assumed that use of the substance carries little risk of an adverse environmental effect. If the PEC/PNEC >1, then a decision must be made either to further refine the data upon which the risk characterisation is based (i.e., progress to a subsequent tier), to manage the risk by limiting the amount of the substance released to the environment or to accept the level of risk following risk-benefit analysis. This latter option may be particularly pertinent to pharmaceuticals. Environmental (and human) risk assessment of both new and existing industrial substances in the European Union are conducted according to the Technical Guidance Document or TGD (CEC, 1996).

By means of a new directive (93/39/EEC), the Council of the European Union amended 65/65/EEC ("*Council Directive 65/65/EEC of 26 January 1965 on the approximation of provisions laid down by law, regulation or administration action relating to medicinal products*"). Article 4.6 of the amendment states "*If applicable, reasons for any precautionary and safety measures to be taken for the storage of the medicinal product, its administration to patients and for the disposal of waste products, together with an indication of any potential risks presented by the medicinal product for the environment*" (93/39/EEC). The amendment effectively requires an environmental risk assessment (ERA) be submitted with marketing authorisation applications (MAA) for pharmaceutical products containing novel compounds. EU member states were required to implement necessary regulations by January 1st, 1995.

However, technical guidelines for the Environmental Risk Assessment (ERA) of pharmaceutical products for human use have yet to be finalised and are still subject to change. Drafts were prepared in 1994-95 under the aegis of several technical committees, including a task force under DG III (the European Commission's Industry Directorate) providing comment to the CPMP ("*Committee for Proprietary Medicinal Products*"). The nature and implications of the draft European guidelines from 1994-1995 are more fully reviewed elsewhere (see Hussain and Hennessy, 1995; Olejniczak, 1995; Webb, 1995). Development of the EU guidance was halted pending clarification and further information from the US Food and Drug Administration (FDA) with respect to their experience of the value of environmental assessments (EAs). This followed the FDA's declaration that EA requirements for pharmaceuticals were to be simplified and that the number of EAs required to be submitted to the FDA for review would be reduced (FDA, 1995). This conclusion was based on the fact that virtually all EAs submitted to the FDA have been issued with a "*Finding Of No Significant Impact*" (FONSI). Following finalisation of the FDA's EA requirements, there is now a categorical exclusion for New Drug Actives (NDA) if the estimated concentration of the substance at the point of entry into the aquatic environment (i.e., in sewage effluent) is below 1 µg/l (US FDA Final Rule - Federal Register 29/07/97 Vol 62 No 145 p40569-40600). This corresponds to a *de facto* threshold of ~41 t/a in the USA. Guidance on the FDA's EA requirements can be found in an FDA-CDER publication entitled: "*Guidance for Industry - Environmental Assessment of Human Drugs and Biologics Applications*" (FDA-CDER, 1998). Recently, a discussion paper on EU guidelines has been circulated by the EU Committee for Proprietary Medicinal Products (CPMP, 2001). Proposed guidelines are essentially similar to those proposed in 1994-1995. The action limit in the aquatic environment is 0.01 µg/l. This corresponds to a *de facto* threshold of 3 tonnes/annum within the EU before ecotoxicity data will be required. Commentary on the CPMP proposal has been provided in the form of an opinion of the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE, 2001).

This study brings together collated data relating to the usage and ecotoxicity of existing pharmaceuticals. Where possible, these data were employed in preliminary ERAs of compounds in the aquatic compartment (which is assumed to be predominantly relevant) in a fashion consistent with that prescribed by the draft European Guidelines. The intention is to provide perspective that will prove useful during the further development of any assessment criteria.

METHODS

In general, there is a paucity of readily accessible data relating to the consumption of pharmaceuticals. This has hitherto precluded attempts at the systematic analysis of the potential impacts of pharmaceuticals upon the environment. The most comprehensive survey of drug usage to date was conducted by Richardson and Bowron (1985), who examined drug prescription patterns in the UK for the years 1975-76 (see Table 1). Of a total of 1,600 compounds considered, approximately 170 were used at >1 tonne/annum. Of these 170, it is possible to derive consumption values for 141 compounds from the publication. The distribution of the data is summarised in Table 2. The corresponding overall consumption totals <6,700 tonnes/annum.

Usage data for 10 drugs in Germany (1995) is presented in Ternes (1998). Some limited drug consumption data for Sweden, the Netherlands and Denmark are presented in Eckerman and Martineus (1997), Van Der Heide and Hueck-Van Der Plas (1984) and Halling-Sørenson *et al.*, (1998). Within the EU there are almost 2000 pharmaceutical manufacturers operating at up to 400 sites. The number of pharmaceutical preparations produced and sold within the EU is estimated at up to 10,000 (CEC, 1992).

Table 1. - Profile of highest use compounds (derived from Richardson & Bowron, 1985).

Rank	Drug	Category	UK Use (t/a)[12]
1	Paracetamol (Acetaminophen)	Analgesic; Anti-pyretic	2,329
2	Aspirin	Analgesic; Anti-pyretic; Anti-inflammatory	1,103
3	Methyldopa	Anti-hypertensive	120
4	Ibuprofen	Anti-inflammatory	65
5	Benorylate	Analgesic; Anti-inflammatory; Anti-pyretic	63
6	Karaya Gum	Cathartic	63
7	Ampicillin	Anti-bacterial	54
8	Sulphamethoxazole	Anti-bacterial; Anti-pneumocystis	49
9	Oxytetracycline	Anti-bacterial	46
10	Clofibrate	Anti-hyperlipoproteinemic	43
11	Dimethicone	Anti-flatulent	30
12	Inositol Nicotinamide	Vasodilator	26
13	Dextroproproxyphene	Narcotic analgesic	22
14	Tetracycline	Anti-amebic; Anti-bacterial; Anti-ricketettsial	20
15	Meprobamate	Anxiolytic	18

[12] Based on the assumption that use of a drug at the level of 1 tonne/annum in the UK corresponds to a maximum predicted concentration of ~0.15 µg/l in the River Lee. For example, quoted proprietary use of Aspirin at 1,000 tonnes/annum corresponds to a predicted concentration of 146 µg/l. All other predicted river water concentrations are in multiples of 0.146 µg/l (Richardson *pers. comm.*).

Table 2. - Summary of UK consumption data (derived from Richardson & Bowron, 1985).

Tonnes/annum	1 - 10	10 - <20	20 - <30	30 - <40	40 - <50	50 - <100	> 100	Total ≥1
Number	115	13	3	0	3	4	3	141
Frequency (%)	82	9	2	0	2	3	2	100

Given the absence of recently published usage data, an audit of 1995 UK pharmaceutical usage was commissioned from IMS (pharmacies, dispensing general practitioners and hospital pharmacies. Intercontinental Medical Statistics - UK and Ireland Ltd). Approximately 60 compounds were selected for audit on the basis of the availability of ecotoxicity data (thereby allowing risk characterisation).

The results of the audit are presented in Table 4 and reflect sales of all products containing these compounds (irrespective of salt and including combination products) into retail Data relating to the UK usage of OTC analgesics were obtained from the Paracetamol Information Centre (Brandon *pers. comm.*)[13] and the European Aspirin Foundation (Hopkins *pers. comm.*)[14]. Data are summarised in Table 3. Where data are available for both drug consumption and ecotoxicity, an aquatic risk characterisation was undertaken for human pharmaceuticals. A review of short-term/acute ecotoxicity data for macro-invertebrates, fish and algae for over 100 human pharmaceuticals is presented in Chapter 3 (Webb, 2001).

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[13] 1950 - 2000 tonnes. Based on 3.9 - 4.0 billion (10^9) tablet equivalent units of 500 mg (Paracetamol Information Centre).

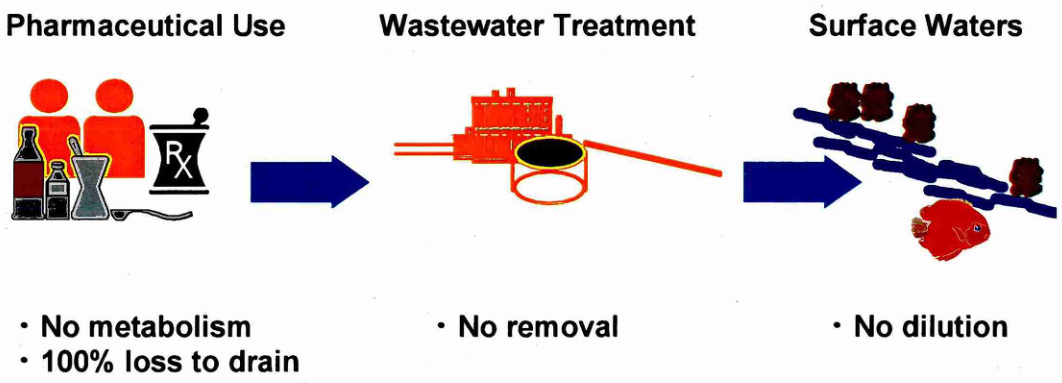
[14] 770 tonnes. Declining sales of Aspirin can be attributed to the increased availability of other OTC analgesics such as Ibuprofen (European Aspirin Foundation).

Table 3. - Summary of 1995 UK consumption data [commissioned from IMS].

Tonnes/annum	<1	1 - 10	10 - <20	20 - <30	30 - <40	40 - <50	50 - <100	> 100
Number	38	14	3	3	1	1	4	3
Frequency (%)	57	21	4.5	4.5	1.5	1.5	6	4.5

Although the results of the risk characterisation are specific to the United Kingdom, they are probably equally applicable to other countries with similarly developed healthcare provisions and wastewater treatment infrastructure. Sewage influent concentrations are calculated throughout on the basis of a UK population of 57.6 million and a specific water consumption of 259 litres/capita/day (WSA, 1994). The assumptions of no human metabolism, passage of all material to drain, no removal during wastewater treatment (via biodegradation, sorption or volatilisation) and no surface water dilution of effluent that were used to calculate the PEC are all conservative. Collectively, they can be thought of as "*worst-case*" (see Figure 2). The PNEC values are derived using an assessment factor of 1,000 with the relevant acute data. This is consistent with the approach employed for other chemical compounds in the EU Technical Guidance Document (CEC, 1996).

Figure 2. - "*Worst-case*" PEC estimation for pharmaceuticals



RESULTS

The results of the preliminary assessment for over 60 compounds are presented in Table 4 and summarised in Figure 3[15]. Together, these compounds will probably account for over half of all known pharmaceuticals consumption in tonnage terms. The PEC/PNEC ratio was <1 in all but eight cases. PEC/PNEC ratios less than unity are taken as indicative of a negligible risk of an adverse environmental effect. The exceptions were Paracetamol (Acetaminophen), Aspirin, Dextropropoxyphene, Fluoxetine, Oxytetracycline, Propranolol, Amitriptyline and Thioridazine. It is notable that Paracetamol and Aspirin are the two most commonly consumed pharmaceutical compounds. One possible caveat to the approach adopted is the implicit assumption of homogenous distribution of use in the UK. Certain drugs such as antineoplastics may only be used in hospitals on an in-patient basis. Further refinement of the risk assessment is therefore required for Paracetamol, Aspirin, Dextropropoxyphene, Fluoxetine, Oxytetracycline, Propranolol, Amitriptyline, and Thioridazine. Chronic ecotoxicity data -which would have allowed use of an alternative assessment factor to calculate the PNEC - are not available for most of the drugs and the initial effects assessments were therefore retained.

Table 4. - Initial aquatic risk assessment for selected pharmaceuticals in the UK.

Name	UK Use (t/a)[16]	PEC (µg/l)	PNEC (µg/l)[17]	PEC/PNEC
Paracetamol	~2,000	367.3	9.2	**39.92**
Aspirin	770	141.4	141	**1.00**

[15] These results were originally presented at SETAC-Europe 1998 (Webb, 1998).

[16] Quoted figures are all assumed to refer to the organic parent molecule (although in some cases the active will actually be the salt and the values will therefore be overestimates).

[17] Asterisk denotes adjustment for molar equivalent of organic parent molecule.

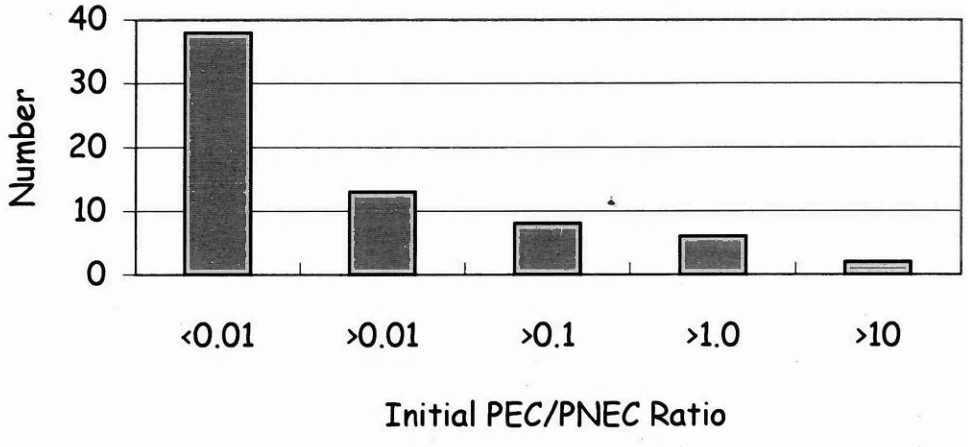
Metformin	106.1	19.49	101*	0.19
Cimetidine	72.0	13.22	740	0.02
Ranitidine	69.0	12.67	582*	0.02
Erythromycin	67.7	12.43	>74	<0.17
Naproxen	60.6	11.13	128*	0.09
Dextropropoxyphene	42.5	7.81	3.79*	**2.06**
Oxytetracycline	33.7	6.19	0.23	**26.8**
Quinine	29.7	5.45	10.1*	0.54
Theophylline	21	3.86	155	0.02
Lithium Salts	20.5[18]	0.35 (Li)	4.18 (Li)	0.08
Metronidazole	15.5	2.85	12.5	0.23
Iopromide	11.9	2.19	>92	<0.01
Propranolol	11.8	2.17	1.87	**1.16**
Verapamil	9.9	1.82	5.78*	0.31
Amitriptyline	5.5	1.01	0.78	**1.29**
Tetracycline	4.7	0.86	16.0	0.05
Omeprazole	3.9	0.72	88	<0.01
Thioridazine	3.8	0.70	0.27*	**2.59**
Chloroquine	2.9	0.53	2.72*	0.20
Gabapentin	2.6	0.48	>1100	<0.01
Etidronic Acid	2.1	0.39	3.0	0.13
Fluoxetine	2.0	0.37	0.026*	**14.19**
Phenobarbital	1.7	0.31	484	<0.01

[18] Includes 16.89 tonnes/annum Lithium Citrate and 3.54 tonnes/annum Lithium Carbonate. PEC and PNEC values are adjusted to the Lithium ion.

Tramadol	1.7	0.31	64*	<0.01
Clofibrate	1.5	0.28	12.0	0.02
Paroxetine	1.3	0.24	1.8*	0.13
Orphenadrine	1.1	0.20	3.82*	0.05
Diazepam	0.957	0.18	4.3	0.04
Acarbose	0.918	0.17	>1000	<0.01
Isoniazid	0.690	0.13	24.4	<0.01
Nefazodone	0.618	0.11	*6.5	0.02
Quinidine	0.601	0.11	7.2*	0.02
Sumatriptan	0.521	0.10	207*	<0.01
Aminosidin/Neomycin E	0.487	0.09	340*	<0.01
Warfarin	0.476	0.09	12.0	<0.01
Lansoprazole	0.434	0.08	18.0	<0.01
Cisapride	0.413	0.08	1000	<0.01
Chloramphenicol	0.377	0.07	305	<0.01
Famciclovir	0.286	0.05	820	<0.01
Azithromycin	0.276	0.05	120	<0.01
Cetirizine	0.273	0.05	278*	<0.01
Famotidine	0.246	0.05	398	<0.01
Ceftibuten	0.095	0.017	>520	<0.01
Lorsatan	0.087	0.016	331	<0.01
Budesonide	0.081	0.015	>19	<0.01
Finasteride	0.067	0.012	20	<0.01
Perindopril	0.047	0.009	>990*	<0.01
Didanosine	0.039	0.007	>1021	<0.01

Midazolam	0.037	0.007	0.2	0.04
Fluticasone	0.034	0.006	0.48*	0.01
Digoxin	0.031	0.006	24	<0.01
Ethinyl Oestradiol	0.029	0.005	0.84	<0.01
Risperidone	0.021	0.004	6	<0.01
Atropine	0.016	0.003	221*	<0.01
Carvedilol	0.008	0.001	1	<0.01
Salmeterol	0.007	0.001	20	<0.01
Bicalutamide	0.007	0.001	>1	<0.01
Alendronic Acid	0.007	0.001	0.46*	<0.01
Dorzolamide	0.004	0.001	604*	<0.01
Diethystilbestrol	0.002	<0.001	1.09	<0.01
Paclitaxel	0.001	<0.001	0.74	<0.01
Zalcitabine	<0.001	<0.001	>1790	<0.01
Thiotepa	<0.001	<0.001	546	<0.01
Flumazenil	<0.001	<0.001	>500	<0.01
Milrinone	<0.001	<0.001	223*	<0.01

Figure 3. - Initial preliminary risk characterisation.



Refinement was consequently confined to the exposure assessments (i.e., PECs). This required a consideration of the likely degree of removal during wastewater treatment (via biodegradation[19], adsorption or volatilisation) and/or surface water dilution of sewage effluent. From a regulatory perspective, any further discussion of removal during wastewater treatment of Aspirin, Dextropropoxyphene, Propranolol, Amitriptyline and Thioridazine is academic as a consideration of surface water dilution alone is sufficient to ensure a PEC/PNEC <1. This assumes a dilution factor 10, which is the default from the EU Technical Guidance Document (CEC, 1996).

Paracetamol: A high degree of elimination (98%) of Paracetamol during activated sludge wastewater treatment has been reported by Ternes (1998). This is not surprising given the biodegradation profile of Paracetamol (Richardson and Bowron, 1985). An estimated Paracetamol "*worst-case*" (i.e., no human metabolism) sewage influent concentration of 367 µg/litre would therefore be reduced to an effluent concentration of 7.3 µg/l following activated sludge treatment. The corresponding PEC after 1-in-10 dilution of effluent in surface water would be 0.7 µg/l. A PNEC of 9.2 µg/l can be derived for Paracetamol by the application of an assessment factor of 1,000 to the lowest of 3 acute endpoints in algae, fish and *Daphnia* (Henschel *et al.*, 1997; Kühn *et al.*, 1989). The resultant PEC/PNEC ratio is 0.08 and environmental safety is assumed (even without any consideration of human metabolism). It is notable that Paracetamol was not detected in the environmental matrices examined by

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[19] Biodegradation data were available for five of the compounds (or a closely related analogue in the case of Oxytetraceycline). The likely biodegradation profile of all the compounds was also predicted using the group contribution method of Boethling *et al.*, (1994). Biodegradation of Paracetamol, Aspirin, Oxytetracycline and Amitriptyline as predicted via the group contribution method was comparable with that reported by Richardson & Bowron (1985). Only in the case of Dextroproproxyphene did reported biodegradation differ from the predicted profile. The group contribution algorithm suggests that Dextropropoxyphene is not recalcitrant and may even be readily biodegradable.

Richardson and Bowron (1985) or in German surface waters (LOD 0.15 μg/l) by Ternes (1998). Concentrations in treated sewage effluents in Germany were similarly less than the detection limit (LOD 0.5 μg/l) at the 90th-percentile (Ternes, 1998). Risk characterisation based the measured (rather than predicted) environmental concentrations (MEC) from Ternes (1998) would yield MEC/PNEC ratios <0.02.

Fluoxetine: The likely biodegradation profile of Fluoxetine can be predicted using the group contribution method of Boethling *et al.*, (1994). The linear model predicts rapid biodegradation. A degree of elimination via biodegradation of Fluoxetine during wastewater treatment is therefore likely. A removal of ≥91% can be predicted for readily biodegradable substances (regardless of K_{ow}) from the WWTP removal defaults in the appendices of the TGD (CEC, 1996). Incorporation of such an elimination rate and a consideration of surface water dilution of effluent (dilution factor 10) would yield a revised PEC for Fluoxetine of 0.003 μg/l. The initial PNEC for Fluoxetine of 0.026 μg/l is derived from an application of an assessment factor to the lowest (algae) of three acute endpoints (FDA-CDER, 1996). A revised PEC/PNEC based upon the above exposure and effects scenarios would be 0.12.

Oxytetracycline: Human metabolism of Oxytetracycline is limited (Dollery, 1991). Biodegradation is also likely to be limited (based on observations for Tetracycline from Richardson and Bowron, 1985). As such the initial assumptions of 100% loss to drain (i.e., no metabolism) and 0% removal during wastewater treatment is retained. The estimated sewage influent and effluent concentrations remain at 6.2 μg/l. With surface water dilution of effluent (dilution factor 10) this corresponds to a PEC of 0.62 μg/l. A PNEC of 0.23 μg/l can be derived from the use of an application factor of 1,000 on an algal EC_{50} (Holtzen-Lützhøft *et al.*,1998). The revised PEC/PNEC is 2.7 and nominally requires further refinement. It is notable that Tetracycline is photodegradable in surface waters with a half-life in terms of hours (Peterson *et*

al., 1993). Photodegradation may help to explain why Oxytetracycline was not detected (90%-tile <LOD 0.05 µg/l) in German rivers by Hirsch *et al.*, (1999). Similarly, the complexing properties of tetracyclines with calcium and other similar ions have also been highlighted as a possible reason to explain their absence from the water column (Hirsch *et al.*, 1999). Risk characterisation based on measured concentrations from Hirsch *et nal.*, (1999) yield MEC/PNEC ratios <0.22.

Ethinyl Oestradiol: Although initial risk characterisation of Ethinyl Oestradiol yielded PEC/PNEC ratios <1, specific concerns have been raised around the possible effects of this compound following observations in environmental matrices. These concerns can also be addressed via risk characterisation based on (i) actual monitoring data rather than predicted concentrations and (ii) a PNEC derived from chronic endpoints. Acute/short term ecotoxicity endpoints for Ethinyl Oestradiol initially employed to derive a PNEC are (i) algal EC_{50} 0.84 mg/l (Köpf, 1995) (ii) *Oncorhynchus mykiss* LC_{50} 1.6 mg/l (Schweinfurth *et al.*, 1996) and (iii) *Daphnia magna* EC_{50} 6.4 mg/l (Schweinfurth *et al.*, 1996). In contrast, sub-lethal responses in *Daphnia* and algae (Köpf, 1995) were at concentrations 1 - 3 orders of magnitude less. Even more sensitive endpoints for Ethinyl Oestradiol exist for fish. They include plasma vitellogenin bioassay effect concentrations of <1 ng/l in rainbow trout (*Oncorhynchus mykiss*) and a spermatogenesis/gonadosomatic index (GSI) effect concentration of 2 ng/l in roach (*Rutilus rutilus*) and rainbow trout (Purdom *et al.*, 1994; FWR, 1995). Although the ecological significance of responses in these biomarkers is unclear, other more readily interpretable endpoints are available. For example, Schweinfurth *et al.* (1996) also detail preliminary chronic test data from studies conducted with fathead minnows (*Pimephales promelas*). These include a 28d LOEC of 10 ng/l for inhibited egg production. A subsequent study revealed a 9-month reproduction NOEC of 1 ng/l (Länge *et al.*, 1997; 2001). With an appropriate assessment factor (i.e., 10), the corresponding PNEC is therefore 0.1 ng/l.

At a consumption figure of 29 kg/annum in the UK - equivalent to ~2.65 million daily doses of 30 μg - the predicted influent concentration is ~5 ng/l. This excludes human metabolism. Less than 1% of Ethinyl Oestradiol is excreted unchanged. The major pathway of metabolism is 2-hydroxylation. Hydroxylated metabolites have little oestrogenic activity. Up to 30% is excreted unoxidised as glucuronide or sulphate conjugates in urine and bile (Dollery, 1991). However, this assumption of no human metabolism is somewhat vindicated by the recent observation that non-oestrogenic steroids metabolites (i.e., Oestradiol-3-glucuronide) can be readily biotransformed into biologically active oestrogens via microbial activity (Panter *et al.*, 1999).

Incorporation of a consideration of removal during waste water treatment (78% by activated sludge treatment and 64% by trickling filter treatment - Ternes *et al.*, 1999) and effluent dilution in surface water (1-in-10) results in a surface water PEC of 0.03 - 0.05 ng/l. This can be compared with values of 2 - 15 ng/l reported in UK rivers by Aherne and Briggs (1989). However, other authorities have doubted the veracity of such observations following subsequent monitoring studies (FWR, 1995). This is supported by the most recent observations from studies detailing (i) German surface water concentrations where values were all <0.2 ng/l (Kalbfus, 1995) and (ii) how Ethinyl Oestradiol was undetectable (<0.2 ng/l) in more than half of UK sewage effluents sampled and where detectable was usually below 1 ng/l (Desbrow *et al.*, 1996). With sufficient surface water dilution of the effluent (i.e., 1-in-10), the PEC would typically range from <0.02 ng/l to <0.1 ng/l, and the environmental safety of Ethinyl Oestradiol could be assumed. Only under low dilution scenarios (i.e., <1-in-2 where effluent concentrations are above the detection limit of 0.2 ng/l) will risk characterisation yield PEC/PNEC ratios >1.

However, it should also be noted that Desbrow *et al.* (1996) also details how the majority (90%) of oestrogenic activity in sewage effluent in the UK is accounted for by the presence of the natural oestrogens, Estrone and 17β-Estradiol. The source of these natural oestrogens appears to be excretion from women, particularly pregnant women. It is suspected that the conjugated forms of Estrone and 17β-Estradiol that are excreted by women are metabolised by bacterial ß-glucuronidase enzymes to produce active hormones. Desbrow *et al.* (1996) therefore concluded that although Ethinyl Oestradiol may contribute to the overall oestrogenicity of sewage effluents, it appears likely that natural oestrogens are responsible for the majority of the feminised responses observed in fish populations exposed to sewage efluents. The corollary of this observation is that additivity can be assumed for steroids. Thorpe *et al.* (2001) demonstrates that binary mixtures of oestrogenic chemicals are additive *in vivo* in juvenile rainbow trout.

Clofibrate/Clofibric Acid: Acute/short term ecotoxicity endpoints for Clofibrate/Clofibric Acid ranged from 12 mg/l to 89 mg/l (Köpf, 1995). The initial PNEC value was 12 µg/l (based on algal EC_{50} from Köpf, 1995) and the PEC was 0.28 µg/l. Risk characterisation yielded a PEC/PNEC ratio of 0.02. For comparison, the PNEC value that can be derived from chronic data is 0.2 µg/l. This is based on a 21d NOEC in *Daphnia magna* of 10 µg/l (Köpf, 1995) with an assessment factor of 50 (algal and *Daphnia* chronic endpoints). The PEC could be further refined by an incorporation of the reported degree of elimination (51%) of Clofibric Acid during wastewater treatment (Ternes, 1998) and surface water dilution of effluent (dilution factor 10). This would yield a PEC of 0.014 µg/l. The maximum reported environmental concentrations (MEC) of Clofibric Acid in surface waters in Germany is 1.75 µg/l with a 90-percentile of 0.72 µg/l (Ternes, 1998). MEC/PNEC ratios are therefore 8.75 and 3.6 respectively. Further revision of the effects assessment to generate a third chronic endpoint for fish would allow an assessment factor of 10. This may improve the MEC/PNEC ratio at the

90-percentile. Differences between the PECs for the UK and reported concentrations of Clofibric Acid in German surface waters in part reflect differing use patterns in the two countries, i.e., 1.5 t/a in the UK compared with 16 t/a reported for Germany by Ternes (1998).

Other Drugs: Observations of surface water concentrations are available for several other pharmaceuticals (see Table 5). Maximal measured environmental concentrations (MECs) for most compounds were generally less than the corresponding "*worst-case*" PECs from Table 3. Although in several cases, the difference was less than one order of magnitude. Whilst many of the measured observations relate to German surface waters, they may be considered indicative of the conservatism employed in this study when deriving the PECs. The surface water concentrations can also be compared with a PNEC to derive MEC/PNEC ratios. In all cases, MEC/PNEC ratios are <1. It is notable that Aspirin, Dextropropoxyphene and Propranolol were also amongst those compounds highlighted for further refinement of the risk assessment following initial characterisation under the "*worst-case*" exposure assessment assumptions (see Table 4).

Table 5. - Comparison of maximal measured environmental concentrations (MEC) with predicted no-effect concentrations (PNEC).

Drug/Metabolite	MEC (ng/l)	PNEC (ng/l)[20]	MEC/PNEC Ratio
Aspirin	340 (i)	141,000	<0.01
Chloroamphenicol	60 (ii)	305,000	<0.01
Dextropropoxyphene	1,000 (iii)	3,790	0.26
Diazepam	<30 (i)	4,300	<0.01

[20] See Chapter 3 (Webb, 2001) for ecotoxicological data for substances marked with an asterisk (*).

Erythromycin	1,700 (ii)	>74,000	<0.02
Ibuprofen	530 (i)	7,100*	0.07
Methotrexate	<6.25 (iv)	85,000*	<0.01
Naproxen	390 (i)	128,000	<0.01
Oxytetracycline	<50 (ii)	230	<0.22
Propranolol	590 (i)	1,870	0.32
Sulfamethazine	<20 (ii)	>100,000*	<0.01
Tetracycline	1,000 (v)	16,000	0.06
Theophylline	1,000 (v)	155,000	<0.01

Source: (i) Ternes (1998); (ii) Hirsch *et al.* (1999); (iii) Richardson & Bowron (1995); (iv) Aherne *et al.* (1985); (v) Watts *et al.* (1983).

DISCUSSION

The general lack of public domain usage data had previously precluded estimates of the environmental concentrations of pharmaceuticals. Data presented in this study allowed the aquatic exposure assessment of a large number of compounds from across a wide variety of therapeutic classes. Initial risk characterisation utilising acute ecotoxicity data and conservative fate assumptions (including no human metabolism, no removal during wastewater treatment and no surface water dilution of effluent) demonstrated the nominal environmental safety (i.e., PEC/PNEC <1) of all but eight of the greater than 60 pharmaceutical compounds considered. The exceptions were Paracetamol, Aspirin, Dextropropoxyphene, Fluoxetine, Oxytetracycline, Propranolol, Amitriptyline, and Thioridazine. Incorporation of their likely fate following use (i.e., removal during wastewater treatment and/or surface water dilution of effluent) yielded a marked reduction in the surface water PEC values, and the resultant PEC/PNEC ratios were generally less than unity when the PNEC was based on acute data. This use of acute ecotoxicity data for risk assessment purposes for pharmaceuticals has

been criticised (Halling-Sørenson *et al.*, 1998). Standard acute bioassays with their focus on immediate endpoints such as lethality may not be the most appropriate basis for risk assessment given the intended narrow scope of biological activity/effect and general potency of pharmaceuticals in general. It has consequently been suggested that chronic bioassays performed over the life-cycle of various organisms from different trophic levels may be more appropriate (Halling-Sørenson *et al.*, 1998). A limited amount of data relating to the effects of pharmaceuticals upon chronic ecotoxicity endpoints was available. It is interesting to note that in this study when PNECs were derived from this chronic data set, values were less than the corresponding values derived from acute data (i.e., in the case of Ethinyl Oestradiol and Clofibrate). MEC/PNEC ratios based on maximal measured surface water concentrations rather than PECs were similarly less than unity. In general, these maximal MECs were less than the "*worst-case*" PECs and this was interpreted as confirmation of the conservative nature of the underlying assumptions used to derive the PECs.

Following use, most human drugs (or their metabolites) will tend to enter the environment by excretion (via urine and/or faeces) from patients. Incorporation of a consideration of human metabolism during the exposure assessment would therefore have the potential to reduce PEC values. Even though it has not proven necessary to include a consideration of metabolism to demonstrate nominal environmental safety of parent compounds, reported metabolism of many drugs is known to be considerable. Where metabolism does take place, metabolites in general will tend to be more polar and water soluble. Metabolism is also often associated with a loss of pharmacological action and detoxification. For example, Richardson and Bowron (1985) noted that a significant number of pharmaceuticals undergo mammalian metabolism to yield conjugates, and that the toxicity and pharmacological activity of these conjugates is likely to be much lower than that of the parent compounds. However, any effects on solubility may also have a concomitant influence on removal via adsorption during wastewater trea-

ment. A full consideration of the fate and effects of all the metabolites of each drug considered in this study would clearly be prohibitive. Risk characterisation was therefore exclusively conducted on the parent drug substance as representative of substances potentially entering the environment. Alternative practices may need to be applied for drugs where there are clear indications that the fate of the metabolites differ from the parent compound or that the metabolites could adversely effect the environment to a greater extent than the parent drug substance. In some cases, incorporation of a consideration of metabolism when estimating exposure may even be inadvisable, as there is a suggestion that some drug conjugates have the potential to be reactivated during biological wastewater treatment (FWR, 1995). Similarly, Henschel *et al.* (1997) speculate whether Paracetamol and Salicylic Acid conjugates can be reactivated via microbial β-gluconidase or sulfatase. Most recently, Panter *et al.* (1999) demonstrated the transformation of a non-oestrogenic steroid metabolite to an oestrogenically active substance via bacterial activity. It is interesting to note that both Clofibrate and Ethinyl Oestradiol - two pharmaceuticals that have attracted particular attention following observations in environmental samples - are excreted in considerable amounts as conjugates in the urine or faeces (Dollery, 1981). Some 50 - 85% of dosed Clofbrate is excreted in the urine as the glucuronic conjugate of Clofibric Acid. In the case of Ethinyl Oestradiol, considerable amounts (~30%) are excreted in urine and bile as the primary glucuronide and sulphate conjugates.

One potential issue that has not been directly addressed is that of bioaccumulation. The potential to bioaccumulate is driven by lipophilicity. Octanol-water partition coefficient (K_{OW}) is a surrogate measure of lipophilicity and has frequently been correlated with bioaccumulation in non-polar/non-ionisable substances, e.g., Mackay (1982) and Veith and Kosian (1983). A quantitative inclusion of a consideration of bioaccumulation into the risk assessment process is described by Cowan *et al.* (1995). One important aspect of the integrated assessment

framework relating to bioaccumulation was whether the duration of ecotoxicity tests are sufficient to achieve maximal body burdens and elicit potential ecotoxic effects upon test organisms. Based on a consideration of such an issue, the study also proposed an initial action threshold for a tiered bioaccumulation assessment of a fish bioconcentration factor (BCF) of 1,000. Both Mackay (1982) and Veith and Kosian (1983) predict a BCF of 1,000 at log K_{ow} ~4.3. Such models typically overestimate actual bioaccumulation and may trigger unjustifiable concerns (ECETOC, 1995). Deviations from predicted bioaccumulation will occur with (i) substances of molecular weight greater than ~700, thereby inhibiting or excluding penetration of biological membranes, (ii) substances that are ionizable, surface active or polar or (iii) where active biotransformation of the substance to a more hydrophilic derivative occurs (ECETOC, 1995).

Of particular importance in reducing the bioconcentration and bioaccumulation of substances within aquatic organisms is biotransformation (ECETOC, 1995). Two types of biotransformation reactions are observed in aquatic organisms and these have been classified as Phase I and Phase II reactions. Phase I reactions are the primary phase of metabolism involving oxidation, reduction or hydrolysis of functional groups. Phase II reactions involve conjugation, whereby substances or their metabolites are bound to other substances such as sulphate or glucuronic acid. Several cases of the influence of metabolism on measured BCFs have been reported, and it is evident that biotransformation is crucial in reducing the bioconcentration and bioaccumulation of substances within aquatic organisms (see ECETOC, 1995). Significant discrepancies can exist between measured and calculated BCF values, and these become more pronounced with increasing log K_{ow} (ECETOC, 1995). Therefore, where biotransformation is known to occur, log K_{ow} cannot be reliably used to predict actual potential to bioconcentrate.

Log K_{ow} data are available for a large number of pharmaceuticals (e.g., Bowman and Rand, 1980; Dollery, 1991; Hansch *et al.*, 1995; Hoekman 1997). Of those considered in this study, only four compounds had log K_{ow} values >4.3. These were Amitriptyline, Chloroquine, Diethylstibestrol and Thioridazine. All have molecular weights <700. It is known that lower vertebrates and invertebrates maintain many of the same systems to biotransform xenobiotics which are present in mammals (ECETOC, 1995). Mammalian metabolism studies may therefore have some value as a first indication of the potential of fish to metabolise a substance. Mammalian metabolism of Diethylstibestrol, Amitriptyline and Thioridazine is similarly reported by Dollery (1991). Only in the case of Chloroquine (K_{ow} 4.63) is mammalian metabolism limited. Excretion is slow with a plasma half-life of 30 - 60 days and Chloroquine may persist in tissues for months or even years after discontinuation of therapy (Reynolds, 1996). Substantial amounts (35%) are excreted unchanged in the urine (Dollery, 1991). However, bioconcentration of Chloroquine is unlikely to be an issue. With pK_a values of 8.4 and 10.8 it will be mainly present as the ionised di-cation moiety at ambient pH in surface waters, and this is likely to limit bioconcentration.

Given that (i) few pharmaceuticals appear to have log K_{ow} values >4.3, (ii) many pharmaceuticals are weak acids/bases and exist as the ionised moiety under conditions of ambient pH, (iii) many pharmaceuticals are readily metabolised to more polar metabolites such as conjugates, (iv) the relatively low levels of pharmaceuticals likely to occur in the environment and (v) the lack of reported examples, it is suggested that the bioaccumulation of human pharmaceuticals will generally not be an issue. This stance is supported by explicit statements from the US FDA that similarly suggest bioaccumulation is not an issue for human pharmaceuticals, i.e.,

> "*In general, pharmaceuticals tend not to be very lipophilic and are produced/used in relatively low quantities compared to industrial chemicals. In humans, the majority*

of pharmaceuticals are metabolized to some extent in humans to SRSs (structurally related substances) that are more polar, less toxic and less pharmacologically active than the parent compound. This suggests that there is a low potential for bioaccumulation or bioconcentration of pharmaceuticals...." (FDA-CEDR, 1998).

"*The vast majority of drugs do not have the physical or chemical characteristics that would allow them to bioaccumulate in tissue because this would raise safety concerns for use in humans. If a drug does have the physical or chemical characteristics that would allow it to bioaccumulate, there has to be some mechanism for the human body to metabolize the compound to a substance that has lower bioaccumulation potential so that it is cleared from the body. In the environmental assessments that CDER reviewed, bioaccumulation has not been an issue.*" (FDA, 1996).

Many pharmaceuticals are weak acids or bases and therefore subject to ionisation (Newton and Kluza, 1978; Raymond and Born, 1986). The degree of ionisation can greatly affect both their fate and the effects as the hydrophobicity, adsorption, volatilisation, bioconcentration, and ecotoxicity of the ionised moiety may differ markedly from the unionised or neutral moiety. These processes will be particularly sensitive to changes in pH in the case of substances with pK_a values within the range of environmentally relevant pHs (i.e., 5-9). This therefore necessitates that due attention be given to the role of ionisation in determining the effects and fate behaviour of pharmaceuticals subject to ERA. The general lack of empirical study in this respect needs to be rectified.

Environmental risk assessment in this study has focused upon the water column and has ignored other compartments such as soil and sediment. Most pharmaceuticals tend to have low K_{ow} values and are often metabolised to more polar (and hence more water soluble) moieties.

As such, the soil and sediment compartments may be less important, although they should not and indeed cannot be ignored.

Given the increasing number of observations of pharmaceutical compounds in sewage, surface waters and drinking water, the environmental fate of pharmaceuticals cannot be ignored and should be considered in the development process. Whilst relative metabolic recalcitrance in humans may be necessary for the pharmacological effect of some compounds (e.g., ethinyl substitution in the case of Ethinyl Oestradiol), it will likely correspond to a poorer biodegradation profile in the environment. As such it may not always be possible to design "*biodegradable*" pharmaceuticals. Under such circumstances, other degradation mechanisms could perhaps be considered. For example, it may be possible to develop photolabile analogues of compounds which otherwise resist metabolism as well as biodegradation *per se*. One example of a drug where photodegradation is known to be the major elimination pathway in surface waters is Diclofenac (Buser *et al.*, 1998; Poiger *et al.*, 2001). Kummerer *et al.*, (2000) similarly highlight how it is potentially feasible to reduce the impact of pharmaceuticals on the aquatic environment by the development of biodegradable structural analogues of existing anti-neoplastic compounds.

CONCLUSIONS

There are a growing number of observations of pharmaceuticals in environmental matrices such as sewage influent, effluent, surface waters and potable water. This implies exposure of aquatic biota and necessitates risk assessment. The general lack of public domain usage data had previously precluded estimates of the environmental concentrations of pharmaceutical. Data presented in this study allowed the aquatic exposure assessment of a large number of compounds (>60) from across a wide variety of therapeutic classes. Risk characterisation

based on acute ecotoxicity data and "*worst-case*" conservative fate assumptions demonstrated the nominal environmental safety (i.e., PEC/PNEC <1) of the large majority of pharmaceuticals considered. For the remainder, the incorporation of their likely fate following use (i.e., likely removal during wastewater treatment and/or surface water dilution of effluent), yielded a marked reduction in the surface water PEC values, and the resultant PEC/PNEC ratios were generally less than unity for most of the compounds. Further refinement of the risk assessment is required for relatively few drugs. An important caveat to this conclusion relates to the assumption that the standard ecotoxicity tests that constitute the acute aquatic database are appropriate to the assessment of compounds with specific modes of action. Risk characterisation was exclusively conducted on the parent drug substance as representative of substances entering the environment. Whilst human metabolism is a mechanism that will potentially reduce environmental exposure, phase II human metabolism of pharmaceuticals to produce conjugates may be reversible if the metabolites are exposed to microbial activity (i.e., in sewage). This potential re-release of biologically active parent compounds has to be considered in any exposure assessment. Metabolites *per se* should also not be ignored if their fate or effects differ markedly from that of the parent compound. The potential bioaccumulation/bioconcentration of pharmaceuticals was also considered, although it was deemed not to be a general issue.

REFERENCES

Aherne GW, Briggs R (1989) The relevance of the presence of certain synthetic steroids in the aquatic environment. *J. Pharm. Pharmacol.* 41: 735-736.

Aherne GW, English J, Marks V (1985) The role of immunoassay in the analysis of micro-contaminants in water samples. *Ecotoxicology and Environmental Safety* 9: 79-83.

Boethling RS, Howard PH, Meylan W, Stitler W, Beuman, J, Tirado N (1994) Group contribution method for predicting probability and rate of aerobic biodegradation. *Environ. Sci. Technol.* 28: 459-465.

Bowman WC, Rand, MJ (1980) *Textbook of Pharmacology* (2nd edition). Blackwell Scientific Publications, Oxford.

Buser H-R, Poiger T, Müller D. (1998) Occurrence and fate of the pharmaceutical drug Diclofenac in surface waters: Rapid photodegradation in a lake. *Environ. Sci. Technol.* 32: 3449-3456.

CEC (1992) *Technical and Economical Study on the Reduction (based on BATNEC) of Industrial Emissions from the Pharmaceutical and Cosmetics Industry.* Commission of the European Communities, Office for Official Publications of the European Communities, Luxembourg.

CEC (1996) *Technical Guidance Document in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EEC) No. 1488/94 on risk assessment for existing substances.* Commission of the European Communities, European Chemicals Bureau, Ispra, Italy.

CPMP (2001) Draft discussion paper on environmental risk assessment of non-gentically modified organism (non-GMO) containing medicinal products for human use. Committee for Proprietary Medicinal Products (CPMP), European Medicines Evaluation Agency (EMEA), London.

Cowan CE, Versteeg DJ, Larson RJ, Kloepper-Sams PJ (1995) Integrated approach for environmental assessment of new and existing substances. *Regulatory Toxicology & Pharmacology* 21: 3-31.

CSTEE (2001) Opinion on draft CPMP discussion paper on environmental risk assessment of medicinal products for human use [non-gentically modified organism (non-GMO) containing] expressed at the 24th CSTEE Plenary Meeting Brussels, June 12th 2001.

Desbrow C, Routledge E, Sheahan D, Waldock M, Sumpter JP (1996) *The identification of oestrogenic substances in sewage treatment effluents.* Report of the Environment Agency, Bristol, U.K.

Dollery CT (ed) (1991) *Therapeutic Drugs - Volume 1 & 2.* Churchill Livingstone, Edinburgh.

ECETOC (1995) *The Role of Bioaccumulation in Environmental Risk Assessment: The Aquatic Environment and Related Food Webs* [Technical Report No. 67]. European Centre for Ecotoxicology & Toxicity of Chemicals, Brussels (available via www.ecetoc.org).

Eckerman I, Martineus JC (1997) *Medicines and the Environment: What do we know today?* A Report from the Swedish Association of Physicians for the Environment (SLFM), Stockholm.

FDA (1995) Streamlining Food & Drug Administration - Environmental Assessments. *FDA Backgrounder* BG95-9 [March 16, 1995].

FDA (1996). 21 CFR Part 25 - National Environmental Policy Act: Proposed Revision of Policies and Procedures; Proposed Rule. *Federal Register* Vol. 61(65) [Wednesday April 3, 1996], 14922-14942.

FDA-CDER (1996) *Retrospective Review of Ecotoxicity Data Submitted in Environmental Assessments [Docket No. 96N-0057].* FDA Center for Drug Evaluation and Research, Rockville (MD), USA.

FDA-CDER (1998) *Guidance for Industry - Environmental Assessment of Human Drugs and Biologics Applications.* July 1998 CMC6 Revision 1. FDA Center for Drug Evaluation and Research, Rockville (MD), USA. [http://www.fda.gov/cder/guidance/index.htm]

FWR (1995) *Effects of Trace Organics on Fish - Phase 2 [July 1995 FR/D 0022].* Foundation for Water Research, Marlow (Bucks.), UK.

Halling-Sørenson B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten-Lützhøft HC, Jørgensen SE (1998) Occurrence, fate and effects of pharmaceutical substances in the environment - A review. *Chemosphere* 36(2): 357-393.

Hansch C, Leo A, Hoekman D (1995) *Exploring QSAR - Hydrophobic, Electronic and Steric Constants.* ACS Professional Reference Book, American Chemical Society, Washington DC (USA).

Henschel KP, Wenzel A, Diederich M, Fliedner A (1997) Environmental Hazard Assessment of Pharmaceuticals. *Regulatory Toxicology & Pharmacology* 25: 220-225.

Hirsch R, Ternes T, Haberer K, Kratz KL (1999) Occurrence of antibiotics in the aquatic environment. *Sci. Tot. Env.* 225: 109-118.

Hoekman D (1997) http://clogp.edu/medchem/chem/clogp/drugs/html [Internet Site].

Holten-Lützhøft HC, Halling-Sorensen B, Jorgensen SE (1998) Algal testing of antiobiotics applied in Danish fish farming. SETAC-Europe 8th Annual Meeting 14-18th April 1998 Bordeaux [Abstract 4I/004].

Hussain Z, Hennessy T (1995) EC environmental risk assessment (1). *The Regulatory Affairs Journal* 6(8): 634 - 640.

Kalbfus W (1995) Belastung bayerischer Gewässer durch synthetische Östrogene. Vortag bei der 50. Fachtagung des Bayerisches Landesamt für Wasserwirtschaft: Stoffe mit endokriner Wirkung im Wasser (Abstract). [Effects in Bavarian watercourses through synthetic oestrogens. Presentation at the 50th Seminar of the Bavarian Association for Waters Supply: Substances with endocrine effects in water (Abstract)].

Köpf W (1995) Wirkung endokriner Stoffe in Biotests mit Wasserorganismen. Vortag bei der 50. Fachtagung des Bayerisches Landesamt für Wasserwirtschaft: Stoffe mit endokriner Wirkung im

Wasser (Abstract). [Effects of endocrine substances in bioassays with aquatic organisms. Presentation at the 50th Seminar of the Bavarian Association for Waters Supply. Substances with endocrine effects in water (Abstract)].

Kühn R, Pattard M, Pernak KD, Winter A (1989) Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to *Daphnia magna*. *Water Research* 23(4): 495-499.

Kümmerer K, Al-Ahmad A, Betram B, Wießler M (2000) Biodegradability of antineoplastic compounds in screening tests: Influence of glucosidation and of stereochemistry. *Chemosphere* 40: 767-773.

Länge R, Schweinfurth H, Croudace C, Panther G (1997) Growth and reproduction of fathead minnow (*Pimephales promelas*) exposed to the synthetic steroid hormone Ethinylestradiol in a life cycle test [Abstract]. 7th Annual Meeting of SETAC-Europe (April 6 - 10, 1997), Amsterdam, The Netherlands.

Mackay D (1982) Correlation of bioconcentration factors. *Environ. Sci. Technol.* 16: 274-276.

Newton DW, Kluza RB (1978) pK_a values of medicinal compounds in pharmacy practice. *Drug Intell. Clin. Pharm.* 12: 546-554.

Olejniczak K (1995) Environmental risk assessment for medicinal products in the EU, Phase I. In: Wolf PU (ed) *Environmental Risk Assessment for Pharmaceuticals and Veterinary Medicines*. Proceedings of the International RCC Workshop held in Basle, Switzerland, February 1 1995. RCC Group, Itingen (Switzerland), pp 58-66.

Panter GH, Thompson RS, Beresford N, Sumpter JP (1999) Transformation of a non-oestrogenic steroid metabolite to an oestrogenically active substance by minimal bacterial activity. *Chemosphere* 38(15): 3579-3596.

Peterson SM, Batley GE, Scammell MS (1993) Tetracycline in antifouling paints. *Marine Pollution Bulletin* 26(2): 96 - 100.

Poiger T, Buser H-R, Müller MD (2001) Photodegradation of the pharmaceutical drug diclofenac in a lake: Pathway, field measurements and mathematical modeling. *Environmental Toxicology & Chemistry* 20(2): 256-263.

Purdom CE, Hardiman PA, Bye VJ, Eno NC, Tyler CR, Sumpter JP (1994) Estrogenic effects of effluents from sewage treatment works. *Chemistry & Ecology* 8: 275-285.

Raymond GG, Born JL (1986) An updated pK_a listing of medicinal compounds. *Drug Intell. Clin. Pharm.* 20: 683-686.

Reynolds JEF (ed) (1996) *Martindale - The Extra Pharmacopoeia* [Electronic Version]. The Royal Pharmaceutical Society of Great Britain. Micromedex Inc., Engelwood, CO (USA).

Richardson ML, Bowron JM (1985) The fate of pharmaceutical chemicals in the aquatic environment. *J. Pharm. Pharmacol.* 37: 1-12.

Schweinfurth H, Länge R, Günzel P (1996) Environmental fate and ecological effects of steroidal estrogens. Presentation at the *Oestrogenic Chemicals in the Environment* conference organised by IBC Technical Services Ltd in London on the 9th & 10th May, 1996.

Ternes TA (1998) Occurrence of drugs in German sewage treatment plants and rivers. *Water Research* 32(11): 3245-3260.

Ternes TA, Stumpf M, Mueller J, Haberer K, Wilken R-D, Servos M (1999) Behaviour and occurrence of estrogens in municipal sewage treatment plants - I. Investigations in Germany, Canada and Brazil. *Sci. Tot. Env.* 225: 81-90.

Thorpe KL, Hutchinson TH, Hetheridge MJ, Scholze M, Sumpter JP, Tyler CR (2001) Assessing the biological potency of binary mixtures of environmental estrogens using vitellogenin induction in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environmental Science & Technology* 35(12): 2476-2481.

Van Der Heide EF, Hueck-Van Der Plas EH (1984) Genessmiddelen en milieu. *Pharmaceutisch Weekblad* 119: 936-947.

Veith GD, Kosian P (1983) Estimating bioconcentration potential from octanol/water partition coefficients. In: Mackay D, Paterson R, Eisenreich S, Simmons M (eds) *PCBs in the Great Lakes (Chapter 15)*. Ann Arbor Science, Ann Arbor, MI (USA).

Watts CD, Crathorne B, Fielding M, Steel CP (1983) Identification of non-volatile organics in water using field desorption mass spectrometry and high performance liquid chromatography. In: Angeletti G, Bjørseth A (eds) *Analysis of Organic Micropollutants in Water*. D.D. Reidel Publishing Company, Dordrecht, pp 120-131.

Webb SF (1995) Current status of the European guidelines on environmental risk assessment. *ESRA Rapporteur* 2(3): 27.

Webb SF (1998) A data-based perspective on the environmental risk assessment (ERA) of human pharmaceuticals [Abstract]. SETAC-Europe 8th Annual Meeting *Interfaces in Environmental Chemistry and Toxicology: from the global to the molecular level* (April 14-18, 1998): Bordeaux, France.

Webb SF (2001) A data-based perspective on the environmental risk assessment of human pharmaceuticals I - Collation of available ecotoxicity data. In: Kümmerer K (ed) *Pharmaceuticals in the Environment*. (pp 203-219) Springer, Berlin.

WSA (1994) *Waterfacts '94* D. Burnell D (ed). Water Services Association, London.

CHAPTER 5

INDIRECT HUMAN EXPOSURE

This chapter has been published in "*Pharmaceuticals in the Environment – Sources, Fate, Effects and Risks*" (Ed. K. Kümmerer) under the title "*A Data Based Perspective on the Environmental Risk Assessment of Human Pharmaceuticals III – Indirect Human Exposure*" pp 221-230 (Springer, 2001).

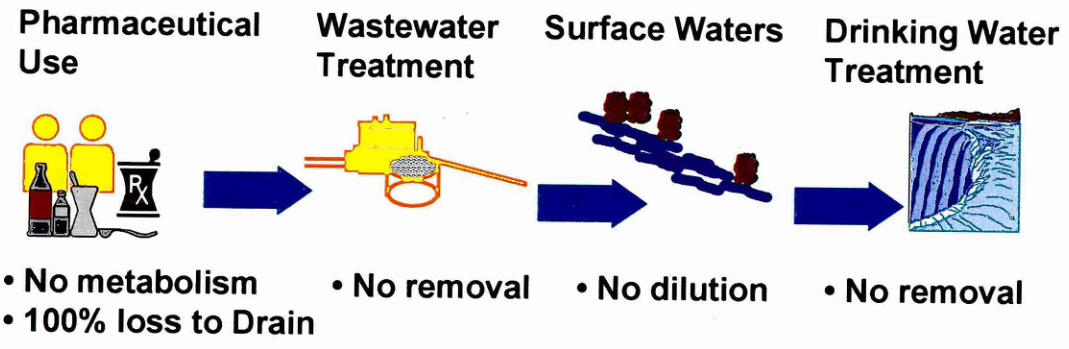
INTRODUCTION

Concerns have previously been expressed over the possibility of adverse human effects arising from indirect exposure to pharmaceuticals via drinking water supplies (e.g., Richardson & Bowron, 1985; Christensen, 1998). This follows numerous observations of pharmaceuticals (or their metabolites) as contaminants in wastewater, surface water and groundwater following normal usage (e.g., Rurainski *et al.*, 1977; Aherne *et al.*, 1985; Aherne & Briggs, 1989; Aherne *et al.*, 1990; Stan *et al.*, 1994; Stumpf *et al.*, 1996; Ternes, 1998; Hirsch *et al.*, 1999). At present there is no regulatory guidance as to how the significance of the potential presence of pharmaceuticals at trace concentrations in drinking water supplies may be assessed. Risk assessment of pharmaceuticals for marketing authorisation purposes within both the United States and European Union do not address this point (Olejniczak, 1995; FDA-CDER, 1998). In order to provide some perspective on this issue, quantitative estimates of potential worse case indirect exposure to pharmaceuticals via drinking water have been undertaken. Potential effects endpoints against which to benchmark such exposure include daily therapeutic dosage.

METHODS

I_{70} values based on the life-time (i.e., 70 years) ingestion of 2 litres/day of water were calculated using the "*worst-case*" predictions for UK surface water concentrations from Chapter 4 (Webb, 2001) with the additional assumption of no drug removal during drinking water treatment (Figure 1). In the absence of a readily available comparable mammalian no-effects endpoints database, the I_{70} values were compared with minimum adult or paediatric daily therapeutic doses (Dollery, 1991; Reynolds 1996). The I_{70} concept was first employed by Richardson & Bowron (1985).

Figure 1. "*Worst-case*" estimation of indirect human exposure to pharmaceuticals via drinking water supplies.



RESULTS

Details of the analysis are presented in Table 1 and summarised in Table 2[21]. The calculated "*worst-case*" lifetime ingestion of a pharmaceutical compound via potable water is of the order of <1 days therapeutic dose for at least 80% of the compounds assessed. For illustration, the calculated "*worst-case*" I_{70} values for Paracetemol, Diazepam and Clofibrate were nine times the daily dose, one and half times the daily dose and one-hundreth of the daily dose respectively. Ethinyl Oestradiol represented the extreme case with a "*worst-case*" I_{70} of 26 times the daily dose.

Table 1. - "*Worst-case*" lifetime drinking water exposure.

Compound	UK Use (t/a)	PEC (µg/l)	Dose (mg/d)	I_{70} (mg)	I_{70} (daily dose)
Paracetamol	~2,000	367.3	2,000 (analgesia)	18769	9.38
Aspirin	770	141.4	1,200 (analgesia)	7226	6.02
Metformin	106.1	19.49	500 (type 2 diabetes)	996	1.99

[21] These results were originally presented at SETAC 1998 (Webb, 1998).

Cimetidine	72	13.22	800 (gastric/duodenal ulceration)	676	0.84
Ranitidine	69	12.67	300 (gastric/duodenal/stomal ulceration)	647	2.16
Erythromycin	67.7	12.43	1,000 (bacterial infection)	635	0.64
Naproxen	60.6	11.13	500 (analgesia)	569	1.14
Dextroproproxyphene	42.5	7.81	175 (analgesia)	399	2.28
Oxytetracycline	33.7	6.19	1,000 (microbial infection)	316	0.32
Quinine	29.7	5.45	1,500 (malaria)	279	0.18
Theophylline	21	3.86	240 (bronchospasm in asthma)	197	0.82
Lithium Salts	20.5	0.35 (Li)	75 (manic depression)	17.9	0.23
Metronidazole	15.5	2.85	1,200 (protozoal infections)	146	0.12
Iopromide	11.9	2.19	20,000 (angiography/urography/ arthrography contrast medium)	112	<0.01
Propranolol	11.8	2.17	80 (hypertension/angina)	111	1.39
Verapamil	9.9	1.82	120 (supraventricular arrhythmia)	93	0.78
Amitriptyline	5.5	1.01	75 (depression)	52	0.69
Tetracycline	4.7	0.86	1,000 (bacterial infection)	44	0.04
Omeprazole	3.9	0.72	20 (duodenal/gastric ulceration)	37	1.84

Thioridazine	3.8	0.7	150 (schizophrenia)	36	0.24
Chloroquine	2.9	0.53	40 (malarial prophylaxis)	27	0.68
Gabapentin	2.6	0.48	<2,400 (treatment of partial epileptic seizures)	25	0.01
Etidronic Acid	2.1	0.39	275 (paget's disease)	20	0.07
Fluoxetine	2	0.37	20 (depressive disorder)	19	0.95
Phenobarbital	1.7	0.31	60 (antiepileptic)	16	0.26
Tramadol	1.7	0.31	50 (analgesia)	15.84	0.32
Clofibrate	1.5	0.28	2,000 (type III hyperlipo-proteinaemia)	14.31	0.01
Paroxetine	1.3	0.24	20 (depression)	12.26	0.61
Orphenadrine	1.1	0.2	150 (parkinsonism)	10.22	0.07
Diazepam	0.957	0.18	6 (insomnia/anxiety)	9.20	1.53
Acarbose	0.918	0.17	300 (type I/II diabetes)	8.69	0.03
Isoniazid	0.69	0.13	300 (tuberculosis)	6.64	0.02
Nefazodone	0.618	0.11	400 (depression)	5.62	0.01
Quinidine	0.601	0.11	500 (atrial fibrillation)	5.62	0.01
Sumatriptan	0.521	0.1	6 (migraine)	5.11	0.85
Aminosidin/ Neomycin E	0.487	0.09	1,500 (intestinal amoebiasis)	4.60	<0.01
Warfarin	0.476	0.09	3 (thrombo-embolic disor-ders)	4.60	1.53
Lansoprazole	0.434	0.08	30 (peptic ulcer)	4.09	0.14
Cisapride	0.413	0.08	30 (gastro-oesophageal re-flux disease)	4.09	0.14

Chloramphenicol	0.377	0.07	3,000 (bacterial infection)	3.58	<0.01
Famciclovir	0.286	0.05	750 (genital herpes & herpes zostera)	2.56	<0.01
Azithromycin	0.276	0.05	1,000 (Chlamydia infection)	2.56	<0.01
Cetirizine	0.273	0.05	10 (hypersensitivity)	2.56	0.26
Famotidine	0.246	0.05	40 (duodenal ulceration)	2.56	0.06
Ceftibuten	0.095	0.017	400 (urinary/respiratory tract infection)	0.87	<0.01
Lorsatan	0.087	0.016	50 (hypertension)	0.82	0.02
Budesonide	0.081	0.015	0.4 (asthma)	0.77	1.92
Finasteride	0.067	0.012	5 (benign prostatic hyperplasia)	0.61	0.12
Perindopril	0.047	0.009	2 (hypertension)	0.46	0.23
Didanosine	0.039	0.007	400 (HIV infection)	0.36	<0.01
Midazolam	0.037	0.007	15 (hypnotic)	0.36	0.02
Fluticasone	0.034	0.006	0.5 (asthma prophylaxis)	0.31	0.61
Digoxin	0.031	0.006	0.125 (congestive heart failure)	0.31	2.48
Ethinyl Oestradiol	0.029	0.005	0.010 (menopausal symptoms)	0.26	26
Risperidone	0.021	0.004	4 (schizophrenia/ psychoses)	0.20	0.05
Atropine	0.016	0.003	0.2 (gastrointestinal disorders)	0.15	0.03
Carvedilol	0.008	0.001	25 (hypertension)	0.05	<0.01

Alendronic Acid	0.007	0.001	10 (post menopausal osteoporosis)	0.05	<0.01
Bicalutamide	0.007	0.001	50 (prostatic cancer)	0.05	<0.01
Salmeterol	0.007	0.001	0.1 (chronic asthma)	0.05	0.5
Dorzolamide	0.004	0.001	20 (glaucoma & ocular hypertension)	0.05	<0.01
Diethystilbestrol	0.002	0.001	1 (prostate carcinoma)	0.05	0.05
Paclitaxel	0.001	0.001	350 (malignant neoplasms)	0.05	<0.01
Flumazenil	<0.001	0.001	0.5 (reversal of benzodiazepine-induced sedation)	0.05	0.10
Milrinone	<0.001	0.001	~80 (1.13/kg/day (severe heart failure)	0.05	<0.01
Thiotepa	<0.001	0.001	60 (bladder cancer)	0.05	<0.01
Zalcitabine	<0.001	0.001	2.25 (HIV infection)	0.05	0.02

Table 2. - Summary of "*Worst-case*" lifetime drinking water exposure.

I_{70} (Daily Dose Equivalent)	**I_{70} (Fraction Daily Dose)**	**Number**	**Cumulative Frequency (%)**
>10	$<^1/_{2,500}$	1	1.5
>5	$<^1/_{5,000}$	2	4.5
>1	$<^1/_{25,000}$	10	19.4
>0.5	$<^1/_{50,000}$	10	34.3
>0.1	$<^1/_{250,000}$	13	53.7
>0.05	$<^1/_{500,000}$	4	59.7

>0.01	$<^{1}/_{2,500,000}$	9	73.1
<=0.01	$>^{1}/_{2,500,000}$	18	100
Total	-	67	100

DISCUSSION

Within the European Union, the quality of water for human consumption is determined by the Drinking Water Directive (Council Directive 98/93/EC on the quality of water intended for human consumption). None of the 48 parameters within the directive relate to pharmaceuticals. The most comprehensive consideration of the potential long-term public health risk of the ingestion of drinking water contaminated with human pharmaceuticals was undertaken by Richardson & Bowron (1985). I_{70} values based on the life-time (i.e., 70 years) ingestion of 2 litres/day of water were similarly calculated using "*worst-case*" predictions for surface water concentrations. These I_{70} values were also similarly compared with typical adult and paediatric therapeutic doses. The calculated ingested quantities were small and a lifetime ingestion of a pharmaceutical compound via potable water would typically be of the order of one days recommended therapeutic dose. The calculated I_{70} values for Paracetemol, Diazepam and Clofibrate were four times the daily dose, one daily dose and one-sixth the daily dose respectively. Similar results for I_{70} were observed in this study with large differences between I_{70} values and therapeutic doses. More recently, Christensen (1998) estimated "*worst-case*" environmental fate and human exposure of Ethinyl Oestradiol (oestrogen), Phenoxymethylpenicillin (antibiotic) and cyclophosphamide (antineoplastic) employing the EUSES software (see http://ecb.ei.jrc.it/existing-chemicals). The results yielded a "*negligible*" human risk connected to predicted human exposure based on diffuse emissions from the use phase via drinking water and diet (vegetables, fish, meat and dairy produce). The effects benchmarks were male endogenous oestrogen production, tolerable food residues based an allergic reactions and genotoxic carcinogenicity thresholds respectively.

Relatively few attempts have been made to detect pharmaceuticals in potable water supplies. Data for a number of compounds are presented in Table 3. These include observations for Bleomycin (Aherne *et al.*, 1990), Clofibric Acid (Stan *et al.*, 1994; Heberer *et al.*, 1997), Diazepam (Waggott, 1981), Diethylstilbestrol (Rurainski *et al.*, 1977), Ethinyl Oestradiol (Rurainski *et al.*, 1977; Aherne *et al.*, 1985; Aherne & Briggs, 1989; Kalbfus, 1995; James *et al.*, 1998), Fenofibrate (Heberer *et al.*, 1997), Ibuprofen (Heberer *et al.*, 1997), Methotrexate (Aherne *et al.*, 1985), Norethisterone (Aherne *et al.*, 1985; Aherne & Briggs, 1989), Penicillins (Richardson & Bowron, 1985), Phenazone (Heberer *et al.*, 1997) and Propyphenazone (Heberer *et al.*, 1997).

Table 3. - Observations of human pharmaceuticals in potable water supplies.

Compound	Concentration (ng/l)	Reference
Bleomycin	(i) range <5 - 13 (ii) mean 8.7	Aherne *et al.* (1990)
Clofibric Acid	10 - 165	Stan *et al.* (1994)
Clofibric Acid	70 - 7,300*	Heberer *et al.* (1997)
Diazepam	~10	Waggott (1981)
Diclofenac	<LOD - 380*	Heberer *et al.* (1997)
Diethylstilbestrol	(i) range 0 - 0.8 (ii) mean 0.11 - 0.24	Rurainski *et al.* (1977)
Ethinyl Oestradiol	(i) range 0 - 22.5 (ii) mean 0.69 - 3.18	Rurainski *et al.* (1977)
Ethinyl Oestradiol	< 5	Aherne, English & Marks (1985)
Ethinyl Oestradiol	<1 - 4	Aherne & Briggs (1989)
Ethinyl Oestradiol	<0.2	Kalbfus (1995)

Ethinyl Oestradiol	<0.4	James *et al.* (1998)
Fenofibrate	<LOD - 45*	Heberer *et al.* (1997)
Ibuprofen	<LOD - 200*	Heberer *et al.* (1997)
Methotrexate	< 6.25	Aherne, English & Marks (1985)
Norethisterone	< 10	Aherne, English & Marks (1985)
Norethisterone	<2 - <10	Aherne & Briggs (1989)
"*Penicilloyl Groups*"	< 10	Richardson & Bowron (1985)
Phenazone	<10 - 1,250*	Heberer *et al.* (1997)
Propylphenazone	<LOD - 1,465*	Heberer *et al.* (1997)

* groundwater supply to drinking water treatment plant

One of the studies relating to occurrence of pharmaceuticals in water supplies concerns observations of Clofibric Acid in German potable water. Concentrations ranged from 10 - 165 ng/l (Stan *et al.*, 1994). The corresponding refined I_{70} value based on measured observations would be 0.5 - 8.4 mg. This can be compared to a daily maintenance dose for Clofibrate of up to 2,000 mg to give an I_{70} value expressed as 0.004 days. A similar calculation can be made for Ethinyl Oestradiol on the basis of the most recent observed concentrations of <0.4 ng/l and <0.2 ng/l in UK and German potable water supplies (James *et al.*, 1998; Kalbfus, 1995). This compares to the "*worst-case*" PEC of 5 ng/l employed here. The refined I_{70} value of 0.02 mg can be compared with a minimum daily therapeutic dose of 0.01 mg used in the treatment of menopausal symptoms. This equates to an I_{70} value of 2 days when expressed as daily dose. Although concerns have been expressed over the possibility of adverse effects on human reproductive biology arising from the presence of oestrogenic substances in drinking water (Ginsburg *et al.*, 1994), a review of international water use patterns highlighted a lack

of homogeneity and suggested that drinking water is consequently unlikely to be a significant factor (Fawell & Wilkinson, 1994). The lack of a vitellogenin response on the part of caged fish in UK raw water storage reservoirs, in contrast to sewage effluent discharges, may similarly be interpreted as supporting this conclusion (FWR, 1995). After Ethinyl Oestradiol, the compound with the highest initial "*worst-case*" I_{70} value when expressed as daily dose was Paracetamol (9 days). This ignores a high degree (98%) of elimination during wastewater treatment (Ternes, 1998) and surface water dilution (default 1-in-10) of treated wastewater effluent. Incorporation of these factors would yield a refined PEC of 0.7 µg/l compared to the "*worst-case*" 367 µg/l employed here. Refinement of the I_{70} on this basis would result in a value of 35.8 mg or 0.02 days. The conservatism of the initial "*worst-case*" PEC for Paracetamol is confirmed by the lack of observation of Paracetamol at detectable concentrations in surface waters in the UK (Richardson & Bowron, 1985), Germany (Ternes, 1998) and the Netherlands (Van Hoof *et al.*, 2000).

Also contributing to the discrepancy between observed concentrations of pharmaceuticals in potable water and the "*worst-case*" concentrations employed here will be drinking water treatment processes. For example, Hutchinson *et al.* (1996) details the efficacy of a number of drinking water treatment processes (chlorination, ozonation, coagulation and powdered activated carbon) on a range of steroids on a laboratory scale. Chlorination, ozonation and powdered activated carbon were effective at removing steroids, but coagulation with aluminium sulphate had little effect. A subsequent study (James *et al.*, 1998) employing similar methodologies confirmed the efficacy of chlorination, ozonation and powdered activated carbon (>95% steroidal removal) and the ineffectiveness of coagulation. It additionally, demonstrated that filtration was ineffective but aeration was quite effective. Ternes (2000) similarly confirmed the general efficacy of drinking water treatment for a large number of pharmaceuticals.

In considering the fate of pharmaceuticals, several studies have highlighted cytotoxic drugs such as anti-neoplastics (e.g., Aherne *et al.*, 1985; Richardson & Bowron, 1985; Lee, 1988; Aherne *et al.*, 1990). Many of these are carcinogenic, mutagenic, embryotoxic or teratogenic and concerns have been expressed over potential risks to potable water supplies. However, where observations from environmental samples are available, concentrations of cytotoxic drugs are limited. For example, concentrations of Methotrexate in river water and potable water samples were all found to be <6.25 ng/l. This can be compared to a concentration of 1 µg/l found in a sewer immediately downstream of an oncology clinic (Aherne *et al.*, 1985). Sewage and water treatment, dilution and degradation effectively reduced this level in the river and potable samples. Methotrexate itself is known to be readily metabolised and to undergo hydrolytic decomposition. Bleomycin was chosen for study by Aherne *et al.* (1990) on the basis of its relative stability. Concentrations of this cytotoxic drug varied from 11 - 19 ng/l in effluents to <5 - 17 ng/l in river and potable water samples. Aherne *et al.* (1990) concluded that any risk to public health from such levels of Bleomycin in drinking water was unlikely. This followed the calculation that consumption of 2 litres/day of such water would result in the ingestion of one-millionth of the daily adult dose of 20 - 30 mg/day. Other anti-neoplastics detected in the aquatic environment (but not drinking water) include Ifosamide and Cyclophosphamide (Steger-Hartmann *et al.*, 1996; Kümmerer *et al.* 1997). One major concern with anti-neoplastics is the possibility that a cancer risk may exist at any level of exposure (i.e., there is no threshold dose). In the case of other carcinogenic compounds, mathematical models have been developed to try and predict the hypothetical incremental cancer rate at low doses. These models require the selection of an acceptable cancer risk - typically 1×10^{-5} to 1×10^{-6} (i.e., one-in-one hundred thousand to one-in-one million). Such an approach may be appropriate in determining limits on anti-neoplastics (and other potentially carcinogenic pharmaceuticals) in drinking water supplies. Sources of data relating to the carcino-

genicity (or otherwise) of pharmaceuticals in general include IARC (1974; 1977; 1979; 1980; 1981; 1990; 1996; 1999) and Fung *et al.* (1995).

Implicit in the calculation of I_{70} values is a life-time exposure over 70 years i.e., 25,550 days. For the large majority (80%) of compounds with I_{70} values equivalent to <1 days daily therapeutic dose, this implies a margin of ≥25,000 (2.5×10^4) between indirect exposure and efficacious therapeutic dosage. Refinement of the exposure for the remaining compounds with "*worst-case*" I_{70} values equivalent >1 days daily therapueutic dose would undoubtedly lead to smaller values if based on more realistic fate scenarios or measured concentrations. Witness the reduction in the refined I_{70} value for Ethinyl Oestradiol, Clofibrate or Paracetamol. The relevance of the use of therapeutic dosage as a benchmark can undoubtedly be questioned, but the absence of a readily available comprehensive chronic mammalian NOEL (no-observed-effect level) database for pharmaceuticals obliged its use in this study and by Richardson & Bowron (1995). Similarly, caveats need to be voiced concerning the potential issues associated with potential life-long exposure at low sub-therapeutic levels and what risk assessment paradigm should apply under such circmustances. A sub-set of the population that are potentially exposed to low sub-therapeutic levels of a pharmaceutical over extended periods are workers from the pharmaceutical industry. One approach used to derive occupational exposure limits (OELs) for pharmaceuticals is based on the application of a safety factor to the lowest recommended therapeutic dose in order to determine a therapeutically non-effective dose (Ku, 2000). This safety factor is typically 100. Whilst there are some issues associated with this approach (i.e., cases of compounds where toxicity is unrelated to pharmacological effects or compounds used in life threatening situations where significant toxicities are acceptable), it does offer some useful perspective and perhaps a base from which to derive an acceptable exposure limit for the population as a whole. If an additional safety factor of 10 were applied to OELs in order to derive general population exposure limits, a margin of

safety would still apply to all compounds with I_{70} values <25 days. In the case of an additional safety factor of 100, a margin of safety would apply to all those compounds with I_{70} values <2.5 days.

CONCLUSIONS

Numerous observations of pharmaceuticals (or their metabolites) in wastewater, surface water and groundwater have given rise to concerns over the possibility of adverse human effects arising from indirect exposure to pharmaceuticals via drinking water. In the absence of regulatory guidance as to how the significance of such contamination to human health may be assessed, quantitative estimates of potential life-time "*Worse Case*" indirect exposure to pharmaceuticals via drinking water have been undertaken and benchmarked against daily therapeutic dosage. Calculated "*worst-case*" life-time (70 years) ingestion for pharmaceutical compounds via potable water is <1 day therapeutic dose for at least for 80% of the compounds assessed. This implies a margin of at least 25,000 between indirect exposure and efficacious therapeutic dosage. For compounds where "*worst-case*" life-time ingestion was >1 day therapeutic dose, refinement of the exposure for several compounds (i.e., Paracetamol, Clofibrate and Ethinyl Oestadiol) demonstrated the degree of conservatism associated with the exposure estimations. Overall it appears that indirect exposure to pharmaceuticals via the potable water supply is unlikely to represent a general objective safety issue. It is however, an area that requires further attention.

REFERENCES

Aherne GW, Briggs R (1989) The relevance of the presence of certain synthetic steroids in the aquatic environment. *J. Pharm. Pharmacol.* 41: 735 - 736.

Aherne GW, English J, Marks V (1985) The role of immunoassay in the analysis of micro-contaminants in water samples. *Ecotoxicology and Environmental Safety* 9: 79-83.

Aherne GW, Hardcastle A, Nield AH (1990) Cytotoxic drugs and the aquatic environment: estimation of bleomycin in river and water samples. *J. Pharm. Pharmacol.* 42: 741-742.

Christensen FM (1998) Pharmaceuticals in the environment - A human risk? *Regulatory Toxicology & Pharmacology* 28: 212-221.

Dollery CT (ed) (1991) *Therapeutic Drugs - Volume 1 & 2.* Churchill Livingstone, Edinburgh.

Fawell JK, Wilkinson MJ (1994) Oestrogenic substances in water: a review. *J. Water SRT - Aqua* 43(5): 219 - 221.

FDA-CDER (1998) *Guidance for Industry - Environmental Assessment of Human Drugs and Biologics Applications.* July 1998 CMC6 Revision 1. FDA Center for Drug Evaluation and Research, Rockville (MD), USA. [http://www.fda.gov/cder/guidance/index.htm]

Fung VA, Barrett JC, Huff J (1995) The carcinogenesis bioassay in perspective: application in identifying human cancer hazards. *Environ. Health Perspect.* 103(7-8): 680-683.

FWR (1995) *Effects of Trace Organics on Fish - Phase 2 [July 1995 FR/D 0022].* Foundation for Water Research, Marlow (Bucks.), UK.

Ginsburg J, Okolo S, Prelevic G, Hardiman P (1994) Residence in the London area and sperm density. *Lancet* 343: 230.

Heberer T, Dünnbier U, Reilich C, Stan HJ (1997) Detection of drugs and drug metabolites in groundwater samples of a drinking water treatment plant. *Fresenius Environ. Bull.* 6: 438-443.

Hirsch R, Ternes T, Haberer K, Kratz KL (1999) Occurrence of antibiotics in the aquatic environment. *Sci. Tot. Env.* 225: 109-118.

Hutchinson J, Harding L, Carlile P, Hart J, Fielding M, Kanda R (1996) *Effect of water treatment processes on oestrogenic chemicals.* DW-05 Drinking Water Quality & Health. UK Water Industry Research Limited (UKWIR), London.

IARC (1974) IARC Cancer Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans - Sex Hormones (Volume 6). World Health Organisation International Agency for Research, Lyon, France.

IARC (1977) IARC Cancer Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans - Some Miscellaneous Pharmaceutical Substances (Volume 13). World Health Organisation International Agency for Research, Lyon, France.

IARC (1979) IARC Cancer Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans - Sex Hormones (II) (Volume 21). World Health Organisation International Agency for Research, Lyon, France.

IARC (1980) IARC Cancer Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans - Some Pharmaceutical Drugs (Volume 24). World Health Organisation International Agency for Research, Lyon, France.

IARC (1981) IARC Cancer Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans - Some Antineoplastic and Immunosuppressive Agents (Volume 26). World Health Organisation International Agency for Research, Lyon, France.

IARC (1990) IARC Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans - Pharmaceutical Drugs (Volume 50). World Health Organisation International Agency for Research, Lyon, France.

IARC (1996) IARC Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans - Some Pharmaceutical Drugs (Volume 66). World Health Organisation International Agency for Research, Lyon, France.

IARC (1999) IARC Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans - Hormonal Contraception and Post-Menopausal Hormonal Therapy (Volume 72). World Health Organisation International Agency for Research, Lyon, France.

James HA, Fielding M, Franklin O, Williams D, Lunt D (1998) *Steroid Concentrations in Treated Sewage Effluents and Water Courses - Implications for Water Supplies.* Report Ref. No. 98/TX/01/1. UK Water Industry Research Limited (UKWIR), London.

Kalbfus, W. (1995) Belastung bayerisches Gewässer durch synthetische Östrogene. Vortag bei der 50. Fachtagung des Bayerisches Landesamt für Wasserwirtschaft: Stoffe mit endokriner Wirkung im Wasser (Abstract). [Effects in Bavarian watercourses through synthetic oestrogens. Presentation at the 50th Seminar of the Bavarian Association for Waters Supply: Substances with endocrine effects in water (Abstract)].

Ku RH (2000) An overview of setting occupational exposure limits (OELs) for pharmaceuticals. *Chemical Health & Safety* 7(1): 34-37.

Kümmerer K, Steger-Hartmann T, Meyer M (1997) Biodegradability of the anti-tumour agent ifosamide and its occurrence in hospital effluents and sewage. *Water Research* 31: 2705-2710.

Lee MG (1988) The environmental risks associated with the use and disposal of pharmaceuticals in hospitals. In: Richardson ML (ed) *Risk Assessment of Chemicals in the Environment.* The Royal Society of Chemistry, London, pp 491-504.

Olejniczak J (1995) Environmental risk assessment for medicinal products in the EU, Phase I. In: Wolf PU (ed) *Environmental Risk Assessment for Pharmaceuticals and Veterinary Medicines*. Proceedings of the International RCC Workshop held in Basle, Switzerland, February 1 1995. RCC Group: Itingen (Switzerland), pp 58-66.

Reynolds JEF (ed) (1996) *Martindale - The Extra Pharmacopoeia* [Electronic Version]. The Royal Pharmaceutical Society of Great Britain. Micromedex Inc., Engelwood, CO (USA).

Richardson ML, Bowron JM (1985) The fate of pharmaceutical chemicals in the aquatic environment. *J. Pharm. Pharmacol.* 37: 1-12.

Rurainski R, Theiss HJ, Zimmermann W (1977) Uber das vorkommen von näturlichen und synthetischen oestrogenen im trinkwasser [Concerning the occurrence of natural and synthetic oestrogens in drinking water]. *Gwf-Wasser/Abwasser* 118(6): 288-291.

Stan HJ, Heberer T, Linkerhägner M. (1994) Vorkommen von clofibrinsäure im aquatischen System - Führt die therapeutische Anwendung zu einer Belastung von Oberfllächen-, Grund- und Trinkwasser? - [Occurrence of clofibric acid in the aquatic system - Is the use in human medical care the source of the contamination of surface, ground and drinking water?]. *Vom Wasser* 83: 57-68.

Steger-Hartmann T, Kümmerer K, Schecker J (1996) Trace analysis of the antineoplastics ifosamide and cyclophosphamide in sewage water by two-step solid phase extraction and gas chromatography-mass spectrometry. *Journal of Chromatography A* 726: 179-184.

Stumpf M, Ternes TA, Haberer K, Seel P, Baumann W (1996) Nachweis von arzneimittelrückständen in kläranlagen und fliessgewässern. Vom *Wasser* 86: 291-303.

Ternes, TA (1998) Occurrence of drugs in German sewage treatment plants and rivers. *Water Research* 32(11): 3245-3260.

Ternes TA (2000) Pharmaceuticals: Occurrence in rivers, groundwater and drinking water. In: *Proceedings of International Seminiar on Pharmaceuticals in the Environment* March 9th 2000 (Brussels). Technological Institute (KVIV), Brussels, Belgium.

Van Hoof F, Van Genderen J, Mons M, Claeys C (2000) Perspective of the drinking water industry. In: *Proceedings of International Seminiar on Pharmaceuticals in the Environment* March 9th 2000 (Brussels). Technological Institute (KVIV), Brussels, Belgium.

Waggott, A (1981) Trace organic substances in the River Lee - In: Cooper WJ (ed) *Chemistry in Water Reuse*. Ann. Arbor Publishers Inc., Ann Arbor, MI (USA), pp 55-99.

Webb SF (1998) A data-based perspective on the environmental risk assessment (ERA) of human pharmaceuticals [Abstract]. SETAC 19th Meeting *The Natural Connection: Environmental Integrity and Human Health*. November 15-19, 1998, Charlotte, NC (USA).

Webb SF (2001) A data-based perspective on the environmental risk assessment of human pharmaceuticals II - Aquatic risk characterisation. In: Kümmerer K (ed) *Pharmaceuticals in the Environment*. (pp 203-219) Springer, Berlin.

CHAPTER 6

GENERAL DISCUSSION

AQUATIC EXPOSURE

The general lack of public domain usage data had previously precluded a systematic analysis of the potential impacts of pharmaceuticals. In contrast, data presented in this study have allowed the estimation of environmental concentrations of >60 compounds from across a wide variety of therapeutic classes. When coupled with the available ecotoxicity data, this permitted risk characterisation. The assumptions employed in calculating "*worst-case*" PECs (i.e., no human metabolism, loss of all material to drain, no removal during waste water treatment and no surface water dilution) ensured that estimates were generally conservative. This is confirmed by comparison of MEC values with the PEC values, especially for extensively metabolised and readily biodegradable compounds. The differences tend to be less pronounced for more recalcitrant (i.e., less extensively metabolised/non-biodegradable) compounds such as Tetracycline and Ethinyl Oestradiol. The availability of measured data relating to human pharmaceuticals is increasing rapidly. Over 60 compounds are detailed in Table 3 of Chapter 2. Ternes (1998) alone deals with nearly 40 compounds.

Following use, most human drugs (or their metabolites) will tend to enter the environment by excretion (as urine and/or faeces) from patients. Incorporation of a consideration of human metabolism during the exposure assessment would therefore have the potential to reduce PEC values. Even though it has not proven generally necessary to include a consideration of metabolism in order to demonstrate environmental safety of parent compounds, reported metabolism of many drugs is known to be considerable. Where metabolism does take place, metabolites in general will tend to be more polar and water soluble and therefore less toxic than the parent compounds. For example, Richardson & Bowron (1985) noted that a significant number of pharmaceuticals undergo mammalian metabolism to yield conjugates and that the toxicity and pharmacological activity of these conjugates is likely to be much lower than that of the parent compounds. However, any effects on solubility may also have a concomi-

tant influence on removal via adsorption during wastewater treatment. A full consideration of the fate and effects of all the metabolites of each drug considered here would clearly be prohibitive. In this study, risk characterisation has been exclusively conducted on the parent drug substance (with the exception of Clofibric Acid) as representative of substances entering the environment. Alternative practices may need to be applied for drugs where there are clear indications that the fate of the metabolites differs from the parent compound or that the metabolites could adversely effect the environment to a greater extent than the parent drug substance. In some cases, incorporation of a consideration of metabolism when estimating exposure may even be inadvisable, as there is a suggestion that some drug conjugates have the potential to be reactivated during biological wastewater treatment (FWR 1995; Henschel *et al.*, 1997; Panter *et al.*, 1999). It is interesting to note that both Clofibrate and Ethinyl Oestradiol - two pharmaceuticals that have attracted particular attention following observations in environmental samples - are excreted in considerable amounts as conjugates in the urine or faeces. Some 50 - 85% of dosed Clofibrate is excreted in the urine as the glucuronic conjugate of Clofibric Acid. In the case of Ethinyl Oestradiol, considerable amounts (~30%) are excreted in urine and bile as the primary glucuronide and sulphate conjugates (Dollery, 1981).

AQUATIC EFFECTS

All acute ecotoxicity endpoints considered in this study were >1 µg/l. In a similar exercise, the US FDA Center for Drug Evaluation and Research (CDER) has performed a retrospective review of toxicity information available in EAs previously submitted in support of NDA (FDA-CDER, 1996). These data showed that no observed effects on relevant standard environmental test organisms at drug concentrations below 1 µg/l. Whilst many classes of compound are represented in the acute ecotoxicity database reviewed here, other classes of compounds will not be represented. A general lack of chronic ecotoxicity data was also noted. In

the case of Ethinyl Oestradiol, significant differences in magnitude between acute and chronic endpoints were noted for fish. The acute EC_{50}/chronic NOEC ratio for *Oncorhynchus mykiss* (Rainbow Trout) was 800,000. This observation could be employed as the basis of an argument that would preclude the use of short-term ecotoxicity testing for the purposes of risk assessment of endocrinologically active compounds and oblige chronic testing at least in the context of vertebrates (i.e., fish). Acute/Chronic ratios for *Daphnia* varied from 1 for Iopromide to 1,428 for Clofibrate with a median of ~43 (n = 7). The Acute/Chronic ratio for Ethinyl Oestradiol in Daphnia was 570. These observations do not contrast markedly with A/C ratios in the range of 1.6 to 1,030 (median 22.1) previously reported for invertebrates based on endpoints from the general ecotoxicity database (ECETOC, 1993).

The vast majority of acute ecotoxicity endpoints identified by this study were >0.1 mg/l (with the exception of endpoints for two SSRIs in non-standard bioassays i.e., Fluvoxamine and Fluoxetine). An implied PNEC of 0.1 µg/l can be derived from an acute ecotoxicity endpoint of 0.1 mg/l with an acute assessment factor of 1,000. The corollary is that the risk of adverse environmental effects from these compounds is assumed to be low if the PEC values are <0.1 µg/l. Under the risk assessment paradigm, an environmental concentration threshold of 0.1 µg/l would therefore generally be protective of aquatic biota against the effects of most of the pharmaceuticals considered here.

This can be compared with the "*concentration of no concern*" of 0.01 µg/l in the aquatic compartment promulgated in the draft EU Phase I guidelines in both 1995 (Olejniczak, 1995) and 2001 (CPMP, 2001). Assuming no removal during wastewater treatment and 1 in-10 surface water dilution of effluent, this corresponds to a *de facto* usage threshold of ~3 tonnes/annum in the 15 member state EU (Table 1).

Table 1. - *De facto* drug usage thresholds based on a "*concentration of no concern*" of 0.01 µg/l in the aquatic compartment (from Webb, 1995).

European Union Member States	Population (millions)	Water Consumption (litres/capita/day)[22]	*De Facto* Threshold (kg)
Austria	7.86	261	75
Belgium	10.02	166	61
Denmark	5.15	257	49
Finland	5.05	279	52
France	56.80	225	467
Germany	78.30	199	569
Greece	10.10	200	74
Holland	14.83	213	116
Ireland	3.50	200	25
Italy	57.52	277	582
Luxembourg	0.39	274	4
Portugal	10.40	200	76
Spain	38.81	192	272
Sweden	8.64	291	92
UK	57.60	259	545
EU15	364.97	230	3,065

The EIC ("*Environmental Introduction Concentration*") Tier 0 limit for sewage effluent detailed in the US FDA guidance for environmental assessment of human drugs is 1 µg/l (FDA -

[22] WSA (1994). In the absence of data, a figure of 200 litres/capita/day is assumed for Greece, Ireland and Portugal.

CDER, 1998). With a dilution factor of 10, this corresponds to a surface water concentration of 0.1 µg/l. The limit equates to a *de facto* usage threshold of approximately 40 tonnes/annum in the USA (cf. Europe). There is no regulatory requirement to generate fate and effects data below this threshold. The justification for the limit arose from the retrospective observation (FDA-CDER, 1996). The findings of this study substantiate the experience of the US FDA-CDER in this respect - at least with regard to acute data. The implicit assumption on the part of the FDA that pharmaceuticals will not exert an effect at <0.1 µg/l can obviously be challenged for Ethinyl Oestradiol where the chronic NOEC is 1 ng/l. An empirically based threshold, rather than an arbitrary value, should form the focus for any international horizontal harmonisation of environmental assessment regulations for human pharmaceuticals.

AQUATIC RISK

Following initial "*worst-case*" risk characterisation, only eight compounds had PEC/PNEC ratios >1. In each case, further refinement of the PEC via a consideration of likely fate during wastewater treatment and/or surface water dilution of sewage effluent resulted in a markedly reduced PEC/PNEC ratio and generally lead to an assumption of negligible risk of an environmental effect (i.e., PEC/PNEC <1.0). Further refinement was necessary in the case of Paracetamol, Fluoxetine and Oxytetracycline. In all cases refinement reduced the PEC/PNEC ratios markedly. Earlier caveats about the applicability of acute ecotoxicity data in the context of risk assessment should be repeated here. The only other compounds of any concern highlighted by the study were Ethinyl Oestradiol and Clofibric Acid. In the case of Ethinyl Oestradiol, some potential for an effect in the environment under conditions of low effluent dilution was suggested following a comparison of chronic endpoints with measured effluent concentrations. However, it has been suggested that the oestrogenic activity of sewage effluent is predominantly associated with the presence of natural steroidal oestrogens rather

than Ethinyl Oestradiol (Desbrow *et al.*, 1996). A similar comparison of observed German surface water concentrations of Clofibric Acid with the PNEC also revealed some potential for effect and further refinement (i.e., provision of additional chronic data) was recommended.

The general lack of impacts suggested by the outcome of the risk assessments in this study (albeit based on acute data), mirrors recent US FDA conclusions. Based upon experience in assessing environmental assessments (EA) that accompany the applications for drug approval submitted by industry, the FDA has concluded that it can eliminate the need for EAs in almost all cases (FDA, 1995). This decision is based on that fact that virtually all EAs prepared in recent years have been issued with a "*finding of no significant impact*" (FONSI) by the FDA. Only one product in recent years, Taxol, has been identified as presenting any potentially significant environmental concerns. Moreover, these concerns were unrelated to manufacturing or use and related to harvesting of endangered Pacific Yew trees.

INDIRECT HUMAN EXPOSURE

I_{70} values based on the life-time (i.e., 70 years) ingestion of 2 litres/day of water were calculated using the "*worst-case*" predictions for surface water concentrations. These I_{70} values were then compared with typical adult and paediatric doses. The calculated ingested quantities were small and a lifetime ingestion of a pharmaceutical compound via potable water would typically be of the order of one days recommended therapeutic dose. Prior to this study, the most comprehensive consideration of the potential long term public health risk of the ingestion of drinking water contaminated with human pharmaceuticals was undertaken by Richardson & Bowron (1985) who employed a similar methodology. More recently, Christensen (1998) examined human risk in relation to indirect exposure to Ethinyl Oestradiol, Phenoxymethylpenicillin and Cyclophosphamide from drinking water and diet. The risk was found to be "*negligible*". Other comparisons between measured observations of pharmaceutical compounds

in drinking water and potable water supplies confirmed the conservative nature of the "*worst-case*" exposure calculations. For example, marked reductions in the I_{70} values for Ethinyl Oestradiol and Clofibrate were noted when measured values were employed.

The relevance of therapeutic dosage as a benchmark can be questioned, but the absence of a comprehensive chronic mammalian effects database obliged its use. Similarly, caveats need to be voiced concerning the potential issues associated with potential life-long exposure at low levels and whether the traditional risk assessment paradigm applies under such circumstances. Nevertheless, it intuitively appears that indirect exposure to pharmaceuticals via the potable water supply is unlikely to represent a safety issue. It is however, an area that requires further attention.

BACTERIAL RESISTANCE

One potential indirect effect of drug use not yet highlighted in this study is bacterial resistance to antimicrobials. It is known that the frequency of bacterial strains resistant to antimicrobial agents in the environment can be high (e.g., Ohba *et al.*, 1999). This is attributed to the widespread use of such drugs. Bacteria with the highest level of resistance are typically isolated from selective environments contaminated with anti-microbial agents e.g., sewage effluents and wastewaters from livestock and fish farms. Whilst the debate whether the development of resistance is possible at the concentrations reported in the environment continues, many studies have identified antibiotics in sewage influents, effluents, surface waters and groundwaters (e.g., Hirsch *et al.*, 1999). Hirsch *et al.* (1999) also report how a large proportion of antibiotics are excreted unchanged or as glucuronide conjugates following human administration. The microbial reactivatiion of conjugated antibiotics (Chloroamphenicol & Sulfamethazine) has been reported by Berger *et al.* (1986). These two factors undoubtedly help to explain the occurrence of antibiotics in the environment.

Recently, the possibility of the transfer of resistant genes between bacteria via conjugation and transfer of plasmids in natural environments has attracted particular attention (Kruse & Sørum, 1994; Sandaa & Enger, 1994; McKeon *et al.*, 1996). Calls have been made for some restrictions on the use of anti-microbials with the aim of reducing the frequency of resistance among bacteria in the environment at large (Kruse & Sørum, 1994). The ultimate concern is the reduced efficacy of drug treatment for human and animal diseases caused by resistant pathogens and the resultant public health hazard. Mckeon *et al.* (1996) reports on antibiotic resistance (AR) and multiple antibiotic resistance (MAR) of more than 250 coliform and non-coliform environmental isolates from rural untreated groundwater supplies to sixteen antibiotics. Widespread resistance to several of the antimicrobials considered in the study was observed (i.e., Tetracycline 32%, Chloroamphenicol 16.9%, Neomycin <10% and Sulfisoxazole <10%). More than 90% of the isolates were resistant to at least one of the antibiotics tested. MAR was expressed by 78% of all isolates.

Most recently, Backhaus & Grimme (1999) have presented data on the toxicity of antibiotic agents to the bacterium *Vibrio fischeri* as determined in the chronic bioluminescence inhibition assay (24h) They also discussed the potential for impacts on natural microbial communities following human, veterinary and aquacluture applications of such compounds and speculate that direct effects are to be expected under certain circumstances (e.g., fish farms). Where possible, comparisons can be made between the EC_{10} values obtained by Backhaus & Grimme (1999) and the observations of antibiotic agents in sewage effluents and receiving surface waters made by Hirsch *et al.* (1999). For example, the EC_{10} for Chloroamphenicol is 0.0187 mg/l. This can be compared with reported maximal concentrations of Chloroamphenicol of 0.56 µg/l in effluents and 0.06 µg/l in surface water. The respective MEC/EC_{10} ratios are 0.03 and <0.01. Similarly, an EC_{10} of 0.0046 mg/l for Tetracycline can be compared with

maximal concentrations of <0.05 μg/l in both sewage effluent and surface waters. The resultant MEC/EC_{10} ratios are both 0.01. Assuming that the response of *Vibrio fischeri* is representative of natural microbial communities as a whole, the MEC/EC_{10} ratios (for Chloroamphenicol and Tetracycline) appear to indicate that selective pressure is unlikely in the compartments considered. This may not apply to other situations such as fish farms or veterinary applications, which may result in high local concentrations in sediments or manure.

RISK MANAGEMENT

Medicinal products have historically been exempted from the European legislative framework dealing with the risk management of industrial chemicals. For example, Article 2 paragraph 2(a) of 67/548/EEC (Directive on the approximation of the law, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances) lists "*medicinal products for human or veterinary use, as defined in directive 65/65/EEC...*" as being exempt from the directive. Similarly, Article 1 paragraph 3(a) of 88/379/EEC (Directive on the approximation of the law, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous preparations) *exempts "medicinal or veterinary products as defined by Directive 65/65/EEC...*". Most recently, 94/904/EC (Council Decision of 22 December 1994 establishing a list of hazardous waste pursuant to article 1(4) of Council Directive 91/689/EEC on hazardous waste) does not include unused medicines.

Following the obligation to indicate potential risks presented by medicines to the environment (i.e., via 93/39/EEC) there remains some debate over the form of risk management measures for medicines within the EU. Whilst manufacturers have adequate control over emissions during formulation of product (CEC, 1992), they do not have any control over the emissions resulting from the use and disposal of the product. In the absence of any regulations, they are obliged to persuade suppliers and users to accept and implement relevant risk

management processes. It is notable that no human medicinal product has been refused marketing authorisation on the basis of ecotoxicological risk potential[23]. Indeed, it is unlikely that the current societal paradigm will allow this at present, even though there is increasing intolerance of environmental contamination. Moreover, no mechanism exists whereby environmental risk can effectively be weighed against the value to society from a new or existing medicine. One could therefore argue that while it remains to be decided how the risk management of medicines will take place, all discussions regarding their environmental risk assessment are moot. Accordingly, an extreme stance would be that if no medicine will ever be denied authorisation on environmental grounds, then environmental risk assessment would be unnecessary as the outcome would have no relevance. It is therefore suggested that the nature of a risk management framework for human medicines urgently needs to be addressed before the European environmental risk assessment guidelines are finalised.

Although unlikely in practice, current European legislative provisions do appear to allow an authorisation for a human medicinal product to be declined or revoked on the basis of ecotoxicological risk potential (assuming implementation into respective national legislations). Article 5 of 65/65/EEC (Directive on the approximation of provisions laid down by law, regulation or administrative action relating to proprietary medicinal products) states that "*The authorisation...shall be refused, if after verification of the particulars and documents listed in Article 4, it proves that the proprietary medicinal product is harmful in the normal conditions of use, or that its therapeutic efficacy is lacking or is insufficiently substantiated by the applicant, or that its qualitative and quantitative composition is not as declared*".

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[23] Use of certain anti-neoplastic drugs is restricted to in-patient facilities, although this is probably mainly a result of human safety concerns rather than ecotoxicological risk (see Lee, 1988). It does however remain as one possible option with which to mitigate the environmental effects of a given drug.

A similar provision in Article 11 allows an authorisation to be revoked and states "*the competent authorities of the member states shall suspend or revoke an authorisation to place a proprietary medicinal product on the market where the product proves to be as harmful in the normal conditions of use, or where its therapeutic efficacy is lacking, or where its qualitative and quantitative composition is not as declared. An authorisation shall also be suspended or revoked where the particulars supporting the application as provided for in Article 4 are found to be incorrect...*".

Following 93/39/EEC (Directive amending Directives 65/65/EEC, 75/318/EEC and 75/319/EEC in respect of medicinal products), Article 4 of 65/65 now contains reference to potential environmental risks. Article 4.6 of the amendment *states "If applicable, reasons for an precautionary and safety measure to be taken for the storage of the medicinal product, its administration to patients and for the disposal of waste products, together with an indication of any potential risks presented by the medicinal product for the environment*". It therefore follows that an indication of potential harmful effects arising from environmental exposure or the provision of incorrect data to support environmental safety are sufficient to deny, suspend or revoke human medicinal drug authorisations.

Whilst the debate continues, it is likely that the extent of risk management of human medicines will be limited to the provision of the advice relating to their proper use and disposal via some form of appropriate labelling or other means of hazard identification. Provisions to permit this are already in place Article 2 paragraph 1 of 92/27/EEC (Directive on the labelling of medicinal products for human use and on packaging leaflets) indicates that "*The following particulars shall appear on the outer packaging of medicinal products or, where there is no outer packaging, on the immediate packaging*" and included in the list is "*(j) special pre-*

cautions for disposal of unused medicinal products or waste materials derived from such products, if appropriate". Article 2 paragraph 2 states that "*The outer packaging may include symbols or pictograms designed to clarify certain information mentioned in paragraph 1 and other information compatible with the summary of the product characteristics which is useful for health education...*".

REFERENCES

Backhaus T, Grimme LH (1999) The toxicity of antibiotic agents to the luminescent bacterium *Vibrio fischeri*. *Chemosphere* 38(14) 3291-3301.

Berger K, Petersen B, Buening-Pfaue H (1986) Persistence of drugs occurring in liquid manure in the food chain. *Archive fuer Lebensmittelhygiene* 37, 99-102.

CEC (1992) Technical and economic study on the reduction (based on BATNEC) of industrial emissions from the pharmaceutical and cosmetic industry. Commission of the European Communities. Office for Official Publications, Publications of the European Communities: Luxembourg.

Christensen FM (1998) Pharmaceuticals in the environment - A human risk? *Regulatory Toxicology & Pharmacology* 28: 212-221.

CPMP (2001) Draft discussion paper on environmental risk assessment of non-gentically modified organism (non-GMO) containing medicinal products for human use. Committee for Proprietary Medicinal Products (CPMP), European Medicines Evaluation Agency (EMEA), London.

Desbrow C, Routledge E, Sheahan D, Waldock M, Sumpter JP (1996) *The identification of oestrogenic substances in sewage treatment effluents*. Report of the Environment Agency: Bristol, U.K.

Dollery CT (Ed.] (1991) *Therapeutic Drugs - Volume 1 & 2*. Churchill Livingstone: Edinburgh.

ECETOC (1993) *Aquatic Toxicity Data Evaluation* [Technical Report No. 56]. European Centre for Ecotoxicology & Toxicity of Chemicals: Brussels.

FDA (1995) Streamlining Food & Drug Administration - Environmental Assessments. *FDA Backgrounder* BG95-9 [March 16, 1995].

FDA-CDER (1996) *Retrospective Review of Ecotoxicity Data Submitted in Environmental Assessments [Docket No. 96N-0057].* FDA Center for Drug Evaluation and Research: Rockville (MD), USA.

FDA-CDER (1998) *Guidance for Industry - Environmental Assessment of Human Drug and Biologics Applications.* July 1998 CMC6 Revision 1. FDA Center for Drug Evaluation and Research: Rockville (MD), USA.

FWR (1995) *Effects of Trace Organics on Fish - Phase 2 [July 1995 FR/D 0022].* Foundation for Water Research: Marlow (Bucks.), UK.

Henschel KP, Wenzel A, Diederich M, Fliedner A (1997) Environmental Hazard Assessment of Pharmaceuticals. *Regulatory Toxicology & Pharmacology* 25: 220-225.

Hirsch R, Ternes T, Haberer K, Kratz K-L (1999) Occurrence of antibiotics in the aquatic environment. *Sci Tot. Env.* 225: 109-118.

Kruse H, Sörum H (1994) Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. *Applied & Environmental Microbiology* 60(11): 4015-4021.

Lee MG (1988) The environmental risks associated with the use and disposal of pharmaceuticals in hospitals. In: "*Risk Assessment of Chemicals in the Environment*" (Ed. ML Richardson), 491-504. The Royal Society of Chemistry: London.

McKeon DM, Calabrese JP, Bissonnette GK(1996) Antibiotic resistance gram-negative bacteria in rural groundwater. *Water Research* 29(8): 1902-1908.

Newsome LD, Johnson DE, Lipnick RL, Broderius SJ, Russom CL (1991) A QSAR study of the toxicity of amines to the fathead minnow. In: "*QSAR in Environmental Toxicology - IV*" (Ed. JLM Hermens & A Opperhuizen), 537-551, Proc. of the 4th International Workshop, Veldhoven, The Netherlands, 16 - 20 September 1990. Elsevier: Amsterdam.

Ohba M, Oku M, Kobayashi N, Suzuki T, Nagai M, Abe S, Saito T, Saito K, Enari K, Izaki K (1999) Number of fecal coliforms and occurrence of drug resistant *E. coli* in rivers of Sendai City. *Journal Japan Society on Water Environment* 22(7): 608-616.

Olejniczak K (1995) Environmental risk assessment for medicinal products in the EU, Phase I. In: "*Environmental Risk Assessment for Pharmaceuticals and Veterinary Medicines*" (Ed. PU Wolf), pp 58-66. Proceedings of the International RCC Workshop held in Basle, Switzerland, February 1 1995. RCC Group: Itingen (Switzerland).

Panter GH, Thompson RS, Beresford N, Sumpter JP (1999) Transformation of a non-oestrogenic steroid metabolite to an oestrogenically active substance by minimal bacterial activity. *Chemosphere* 38(15): 3579-3596.

Richardson ML, Bowron JM (1985) The fate of pharmaceutical chemicals in the aquatic environment. *J. Pharm. Pharmacol.* 37: 1-12.

Sandaa RA, Enger Ø (1994) Transfer in marine sediments of the naturally occurring plasmid pRAS1 encoding multiple antibiotic resistance. *Applied & Environmental Microbiology* 60(12): 4234-4238.

Ternes TA (1998) Occurrence of drugs in German sewage treatment plants and rivers. *Water Research* 32(11): 3245-3260.

Webb SF (1995) Current status of the European guidelines on environmental risk assessment. *ESRA Rapporteur* 2(3): 27.

WSA (1994) *Waterfacts '94* (Ed. D Burnell). Water Services Association: London.

CHAPTER 7

SUMMARY

- There are a growing number of observations of pharmaceuticals in environmental matrices such as sewage influent, effluent, surface waters and potable water. This implies exposure of aquatic biota and/or indirect human exposure and necessitates risk assessment.

- Where acute aquatic data are available, the degree of aquatic ecotoxicity of the large majority of pharmaceuticals is limited. This is not unexpected given the low mammalian toxicity that is required of pharmaceuticals in general. All acute ecotoxicity endpoints were >1μg/l. A lack of published ecotoxicity data was noted for certain classes of compounds. Similarly, the chronic ecotoxicity database is limited.

- The general lack of public domain usage data had previously precluded estimates of the environmental concentrations of pharmaceutical. Data presented in this study allowed the aquatic exposure assessment of a large number of compounds from across a wide variety of therapeutic classes. Where available, surface water MEC values were generally less than "*worst-case*" exposure predictions.

- Risk characterisation utilising acute ecotoxicity data and "*worst-case*" conservative fate assumptions (including no human metabolism, no removal during wastewater treatment and no surface water dilution of effluent) demonstrated the apparent environmental safety (i.e., PEC/PNEC <1) of all but 8 of the pharmaceuticals considered. The exceptions were Paracetamol (Acetaminophen), Aspirin, Dextropropoxyphene, Fluoxetine, Oxytetracycline, Propranolol, Amitriptyline and Thioridazine. Incorporation of their likely fate following use (removal during wastewater treatment and/or surface water dilution of effluent but not human metabolism), yielded a marked reduction in the surface water PEC values and the resultant PEC/PNEC ratios were less than unity for most of the compounds.

Further refinement of the risk assessment is required for Paracetamol, Oxytetracycline and Fluoxetine. Risk characterisation was exclusively conducted on the parent drug substance as representative of substances entering the environment. As a result of the risk characterisation it is concluded that there is no basis for assuming widespread environmental effects on the part of pharmaceuticals in general. An important caveat to this conclusion relates to the assumption that the standard ecotoxicity tests that constitute the acute aquatic database are appropriate to the assessment of compounds with specific modes of action. Large Acute/Chronic ratios for certain compounds could be used to argue that chronic ecotoxicity testing with endpoints tailored to the specific modes of action be required in the risk assessment of pharmaceuticals. This would entail obvious implications regarding the cost of testing strategies.

- Human metabolism is a mechanism that will potentially reduce environmental exposure. However, Phase II human metabolism of pharmaceuticals to produce conjugates may be reversible if the metabolites are exposed to microbial activity (i.e., in sewage). This potential release of biologically active parent compound has to be considered in any exposure assessment.

- Many pharmaceuticals are weak acids or bases and therefore subject to ionisation. The degree of ionisation can greatly affect both their fate and the effects as the hydrophobicity, adsorption, volatilisation, bioconcentration and ecotoxicity of the ionised moiety may differ markedly from the unionised or neutral moiety. These processes will be particularly sensitive to changes in pH in the case of substances with pK_a values within the range of environmentally relevant pHs (i.e., 5 - 9). This therefore necessitates that due attention be given to the role of ionisation in determining the effects and fate behaviour

of pharmaceuticals subject to ERA. The general lack of empirical study in this respect needs to be rectified.

- Environmental risk assessment in this study has focused upon the water column and has ignored other compartments such as soil and sediment. Most pharmaceuticals tend to have low Kow values and are often metabolised to more polar (and hence more water soluble) moieties. As such the soil and sediment compartments may be less important, although they should not and indeed cannot be ignored.

- Given that (i) few pharmaceuticals appear to have sufficiently elevated K_{OW} values, (ii) many are weak acids/bases and exist as the ionised moiety under conditions of ambient pH, (iii) many are readily metabolised to more polar metabolites such as conjugates, (iv) relatively low levels are likely to occur in the environment and (v) there is a lack of reported examples, it is suggested that the bioaccumulation of human pharmaceuticals will not generally be an issue and may effectively be ignored in the risk assessment of the majority of compounds.

- Although concerns have been expressed over the possibility of adverse human effects arising from the presence of pharmaceuticals in drinking water supplies, quantitative comparisons reveal that potential lifetime ingestion of pharmaceuticals is limited relative to daily therapeutic doses. Further work based on benchmarking predicted exposure with the mammalian toxicological database rather than therapeutic dosing regimes (which by definition illicit an effect) is desirable.

- Given the increasing number of observations of pharmaceutical compounds in sewage, surface waters and drinking water, the environmental fate of pharmaceuticals cannot be

ignored and should be considered in the development process. Whilst relative metabolic recalcitrance in humans may be necessary for the pharmacological effect of some compounds (e.g., ethinyl substition in the case of Ethinyl Oestradiol), it will likely correspond to a poor biodegradation profile in the environment. As such it may not always be possible to design "*biodegradable*" pharmaceuticals. Under such circumstances, other degradation mechanisms could perhaps be considered. For example, it may be possible to design photolabile analogues of compounds, which resist biodegradation *per se*.

- A consideration of risk management concluded that current European legislative provisions would allow a drug marketing authorisation to be declined, suspended or revoked on the basis of ecotoxicological risk, although in practice this was deemed unlikely. Any risk-benefit consideration relates directly to human health and will undoubtedly be fraught with difficulties. It was therefore suggested that the nature of a risk management framework for human medicines urgently needs to be addressed in addition to the European environmental risk assessment guidelines.